LAWRENCE F. ROBERGE
ID UD1415SBI3331

AIU DOCTORAL DISSERTATION

INTRODUCED SPECIES AS A FORM OF BIOLOGICAL WEAPON

ATLANTIC INTERNATIONAL UNIVERSITY

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INSPIRATION:

-"All wisdom comes from the Lord and with him it remains forever."
SIRACH 1:1 (St. Joseph Edition, New American Bible)

- " Mens Est Clavis Victoriae" (The Mind Is The Key To Victory).
Motto on the crest of the United States Army School of Advanced Military Studies

- "The brick walls are there for a reason. The brick walls are not there to keep us out; the brick walls are there to give us a chance to show how badly we want something. The brick walls are there to stop the people who don't want it badly enough. They are there to stop the *other* people!"
Randy Pausch, The Last Lecture (18 September 2007)

LIST OF ABBREVIATIONS	
3P's	Parasites, Pathogens, and Predators
AM	Arbuscular Mycorrhizas
AMF	Arbuscular Mycorrhizal Fungi
APHIS	Animal and Plant Health Inspection Agency (USDA)
APHIS-PPQ	Animal and Plant Health Inspection Agency-Plant Protection and Quarantine
BC	Before Christ
BIDS	Biological Integrated Detection System
BIOCLIM	BIOCLIMatic (ENM modeling software)
BTWC	Biological and Toxin Weapons Convention
BW	Biological Weapons
BWC	Biological Weapons Convention
C-BW CONOPS	Counter-Biological Warfare CONcept of OPerationS
CDC	Centers for Disease Control (US)
CDFA	California Department of Food and Agriculture
CIA	Central Intelligence Agency (US)
CFU	Colony Forming Units
CpG	Cytosine connected by a Phosphodiesterase bond to Guanine (DNA POLYMER STRAND)
CRP	Commodity/Pathway Risk Potential
CPU	Central Processing Unit
CW	Chemical Weapons
DGPS	Differential Global Positioning System
DHS	Department of Homeland Security (US)
DNA	DeoxyriboNucleic Acid
DOD	Department Of Defense (US)
DTRA	Defense Threat Reduction Agency (US)
EF	Edema Factor (Anthrax)
EICA	Evolution of Increased Competitive Ability
ENM	Ecological Niche Modeling (aka Environmental Niche Modeling)
EPA	Environmental Protection Agency (US)
ERCA	Evolutionary Reduced Competitive Ability
ERMA	Environmental Risk Management Authority (New Zealand)
FAO	Food and Agricultural Organization (United Nations)
FBI	Federal Bureau of Investigation (US)
FEMA	Federal Emergency Management Agency (US)
FMD	Foot and Mouth Disease
GA	Genetic Algorithm
GARP	Genetic Algorithm for Rule-set Prediction (ENM modeling software)
GDP	Gross Domestic Product
GIS	Geographic Information System
GISD	Global Invasive Species Database
GISP	Global Invasive Species Programme
GLONASS	GLObal NAvigation Satellite System (Russia)

GPS	Global Positioning System
HiFIS	High-Fidelity Imaging Spectrometer
HUMINT	HUMan INTelligence
ICBM	InterContinental Ballistic Missile
INTERPOL	International Criminal Police Organization
IR	Infra-Red
LACM	Land Attack Cruise Missile
LIDAR	Light Detection and Ranging
LF	Lethal Factor (Anthrax)
LINUX	UNIX-Like operating system
LRBSDS	Long Range Biological Stand-off Detector System
MOPP	Military Operations Protective Posture
NAFTA	North American Free Trade Agreement
NIS	Non-Indigenous Species
NISIC	National Invasive Species Information Center (US)
NRC	National Research Council
N_T	Threshold host population density
OIE	World Organization for Animal Health (Office International des Epizooties)
OSD	Office of the Secretary of Defense
PA	Protective Antigen (Anthrax)
PC	Personal Computer
PCR	Polymerase Chain Reaction
PIN	Port Information Network (APHIS database)
ProMED	Program for Monitoring Emerging Diseases
R_0	Reproductive number of the parasite (or pathogen)
RAPID	Ruggedized Advanced Pathogen Identification Device
RMA's	Revolution In Military Affairs
RNA	RiboNucleic Acid
ROS	Reactive Oxygen Species
RPV	Remotely Piloted Vehicles
SCUD	NATO reporting name for Soviet army short-range ballistic missile
SEB	Staphylococcal Enterotoxin B
SIS	Secret Intelligence Service –aka MI6 (UK)
TBT	Tropical Bont Tick
TERCOM	TERrain COntour Matching
TOPOFF	TOP OFFicials (US drill)
UAV	Unmanned Air Vehicles
UK	United Kingdom
US	United States
USACDA	United States Arms Control and Disarmament Agency
USAF	United States Air Force
USDA	United States Department of Agriculture
USMC	United States Marine Corps
USSR	Union of Soviet Socialist Republics

UV	UltraViolet
WHO	World Health Organization (United Nations)
WMD	Weapons of Mass Destruction
WW I	World War 1
WW II	World War 2
WP.262	Working Paper 262 from BTWC draft protocol
XML	eXtensible Markup Language

CHAPTER 1-ABSTRACT

INTRODUCED SPECIES AS A FORM OF BIOLOGICAL WEAPON

The proposed research dissertation topic is on biological weapons. The hypothesis is that introduced species (aka invasive species) could be used as a form of biological weapon (BW). The first step of this dissertation would be a brief review of the concepts and history of biological weapons. Also, it is important to note the advantages and disadvantages of biological weapons.

The next component of the dissertation would review the definition of an introduced species as well as a brief survey of historical examples of introduced species. The advantages and disadvantages of introduced species would be discussed. It is important to compare and contrast an infection of a pathogen into a host versus the entry role of an introduced species into an ecosystem. One key point to note is the time delay from the entry until establishment of the organism in the host or ecosystem. Also, some of this success in infection or successful introduction of the species depends on the multiple propagules concept (i.e. the success of an infection or invasion depends on the number of organisms entering the host of ecosystem at that time).

The methods to predict successful invasive candidates would be discussed and examples of various theories and computer software models to analyze introduced species invasions will be reviewed. Previous methods depended on historical data AFTER the invasion had already occurred. Accessing global databases of introduced species could also be used to predict the usefulness of select organisms as biological weapons, but again this information is based on <u>previous historical</u> data of successful invasions. The use of GARP (Genetic Algorithm for Rule-set Prediction) has found promise in predicting the range and effective invasiveness of an organism prior to the actual invasion. Also, the factors favoring introduced species should briefly address the interaction of the introduced species with native flora and fauna or the lack of interaction (e.g. escape of pathogen theories) to help understand the variables favoring introduced species success in the non-native niche.

This research proposal does not ignore the possibility of an aggressor nation state using invasive species BW against a target nation. One approach would be to use invasive species BW

in a Fabian policy (i.e. indirect action to avoid direct attack) to render the target nation weakened by shortages of agricultural commodities or biofuel. This weakening would result in subsequent social strife and attenuation in military security that would allow for the rapid conquest of the target nation by the aggressor nation state.

The targets of a BW attack using introduced species are varied depending on the introduced organism as well as the number of organisms (propagules) used during the invasion (single application or multiple applications). The targets of a BW attack could be food resources (either crops or livestock) in an agricultural BW attack or could be directed at biofuel crops to trigger a biofuel shortage in the target nation. Also, it must be noted that using introduced species as a BW attack against ecosystems or the actual biodiversity of a region or nation could be a target, especially if the attack was initiated by bioterrorists motivated to incite fear and social unrest in the targeted region or nation. Another more familiar BW target to use introduced species against would be humans. The means of a human targeted BW attack could include introduced species appearing as emerging diseases (e.g. Rinderpest), vector borne diseases where the vector is the introduced species (e.g. ticks infected with rickettsia pathogens), or introduced species that create infected commodities that could lead to human disease (e.g. prions leading to spongiform encephalitis, heart water, etc.).

One key issue with BW using invasive species is to differentiate a deliberate attack from a natural outbreak or accidental introduction via commerce. The methods to detect a BW attack include: analysis of the introduced species; the number, location, and distribution of the propagules; evidence of expected or unusual sources of introduction (e.g. ballast water from ships); evidence of smuggled organisms; analysis of GARP for adaptive success of the introduced species for that region; human intelligence of a planned BW attack using the introduced organism; and evidence of culturing of the introduced organism by a nation state or terrorist facility.

The strategies to introduce BW invasive species are varied. These strategies include: smuggling organisms in target nations; aerial dispersal of organisms (e.g. seeds, insects, microbes, viruses, fungal spores); migrating birds or insects; introduction of a vector to transport the introduced organisms and infect target crops or animals; human carriers dispersing organisms by roadsides or open fields or forests; or biocruise-the technique of using cruise missile technology (aka unmanned aerial vehicles) to deliver and disperse BW agents (e.g. virus, fungal spores, bacteria, even insects) at precise targeted sites. Since the flight of the cruise missile is controlled through out the flight and using Global Positioning System (GPS) technology virtually assures accurate delivery to targeted fields, the use of cruise missiles could be the high tech means for a successful BW attack. Also, it must not be overlooked that any introduced organism used as a BW could be genetically engineered to possess such properties as enhanced virulence, resistance to pesticides, insecticides or antibiotics, enhanced reproduction rates, or expression of new toxins or vaccine evading antigens.

The vulnerability of nations to invasive species introduction as well as the risk factors favoring invasive species BW would also be examined. Some of these risk factors favoring a BW attack using invasive species are poor communication between local population and government scientists and decision makers; monoculture of agricultural fields; and presence of favored niches in the targeted areas.

Finally, the summary of the data should help support the hypothesis that introduced species could be used as a form of biological weapon. Whether the targets are humans, agricultural resources (livestock or crops), ecosystems or regional biodiversity, this environmental and technological approach to BW should be discussed and where possible monitored for and banned under the Biological and Toxins Weapons Convention (BTWC).

CHAPTER 2 -HYPOTHESIS

That introduced species (aka invasive species or exotic species) could be used as a form of biological weapon. This application could be used on a broad scale by hostile nations as a strategic weapon or on a smaller scale as a form of bioterrorism by rogue nations or non-state actors (terrorists).

The data to support this hypothesis will be basis for this dissertation.

CHAPTER 3- CONCEPTS OF BIOLOGICAL WEAPONS

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CHAPTER 3. CONCEPTS OF BIOLOGICAL WEAPONS

1. DEFINITIONS AND CONCEPTS

Couch defines a biological weapons attack as "the intentional use by the enemy, of live agent or toxins to cause death and disease among citizens, animals, and plants" (2). Daly states the five important attributes of a biological warfare agent are: High virulence coupled with high host specificity; high degree of controllability; lack of timely countermeasures to the attacked population; ability to camouflage the BW agent with relative ease; and high degree of resistance to adverse environmental forces (3). The variety of biological weapons includes bacterial (e.g. Anthrax, Q fever, Tularemia), viral (e.g. Smallpox, Hemorrhagic Fever, Venezuelan Equine Encephalitis), Fungal (e.g. *Coccidioides immitis*), or toxins (e.g. Ricin, Staphylococcal Enterotoxin B (SEB), or T-2 Mycotoxins) (4, 5, 6, 7, 8). Some weapons have been developed using arthropods as vectors (e.g. Yellow Fever, Plague, or Dengue Fever) (9). For example, some reports of BW by the Japanese during World War II (WW II) included the dispersal of plague infected fleas by air to infect villages in China (10).

Furthermore, new agents of BW have been described as bioregulators. Bioregulators are a diverse set of compounds used to manage a wide variety of physiological processes (i.e. homeostasis), including immune responses, heart rate, blood pressure, temperature, bronchial and vascular tone, muscle contractions, as well as blood chemistry, and consciousness. If these compounds were used in an aerosol or oral route, they could be used by bioterrorists to trigger such reactions as fever, hypoglycemia, shock, disseminated intravascular coagulation, mood alteration, stroke, cardiac failure, arterial thrombosis, or death (11).

Finally, BW can be directed against agricultural targets using various bacterial, viral, and fungal agents (e.g. Anthrax, Foot-And Mouth Disease, Wheat Smut) (12, 13). The reasoning for targeting agriculture is that American agricultural products are a key component of the US national infrastructure which besides including food production, it is the number one contributor to the US trade balance of payments (12). Therefore, attacking the agricultural sector could weaken a nation both internally as well as economically in the global market place.

In the delivery of BW agents, weapon delivery systems had evolved tremendously in the 20^{th} Century. Prior to the 20^{th} century and the development of germ theory, the BW methods were crude and based only on the concept of contagion. Mayer (60) describes the first recorded description of the "contagion concept" in Sumerian cuneiform tablets in the archives of Mari in Sumer. These royal letters forbade people from "infected towns" to venture into "healthy towns" to prevent the spread of the disease to the entire country as well as advising no contact with an infected woman or her cup, chair, or bed to prevent the contracting of her disease (60). The Romans, Greeks, and Persians used corpses of animals to pollute water supplies of their enemies (60). Even in 1863, Confederate General Johnson used corpses of sheep and pigs to pollute the drinking water at Vicksburg (14).

There are limitations to the effectiveness of biological weapons as history has demonstrated. These, limitations are dependent on understanding the pathogen, whether the parties clearly knew the disease was due to a pathogen rather than "poisoned air or bad vapors" as in the pre-Germ Theory times (65, 66). Other factors for the success of the biological weapon were dependent on the understudying of the epidemiology of the pathogen, the host range of the pathogen, and the stability of the pathogen in the air (a concept referred to as aerobiology). Some pathogens were sensitive to ultraviolet light and hence could not be tested (or used) during daytime as the ultraviolet light would kill the pathogens (such as anthrax) quickly. Other agents were unstable outside of water (e.g. cholera) and would be favored to contaminate water supplies. Other agents normally were dependent on arthropod vectors for normal delivery of the agent (e.g. mosquitoes, ticks, fleas) and thus either elaborate delivery means were necessary or the cultivation of the arthropod vector was required.

An excellent example of how a biological weapons attack failed by following these above factors was the Confederate scheme during the spring of 1865 to ship yellow fever contaminated clothing, blankets and sheets to the poor of New York city and other northern cities (59). A confederate ally in Bermuda, Dr. Luke P. Blackburn, collected the materials from Bermudans who were sick and/or died of the disease. Because he had treated many Bermudans sick with the disease, Dr. Blackburn knew of the disease (a disease caused by the flavivirus that is transmitted by the bite of the *Aides aegyptii* mosquito), but he did not know the precise method of infection at the time and did not understand that the contaminated material would not infect the user as General Amherst's strategy with smallpox did one century earlier. The plan nevertheless was thwarted before the contaminated items were shipped to the North and yet Dr. Blackburn was never imprisoned for his plot. (59)

Yet, in the 20th Century, with the identification and isolation of pathogens as well as the techniques to culture the agents, BW munitions (Biomunitions) were developed to disseminate BW agents (4). Biomunitions were either strategic or tactical and their application depended on the following factors: strategic or tactical value, causalities, flexible results, large or small area of coverage, no physical destruction, and cost (4). Most agents were dispersed in the air (aerosol), but even airborne agents had differing effectiveness due to the mode of agent dispersal (dry particle versus wet particle), meteorological conditions (dry air versus rainy weather), temperature (cool evening air versus hot daytime air), daytime or nighttime use (e.g. ultraviolet rays from sunlight would inactivate many agents rather quickly), and number of live cells necessary to cause infection. The United States (US) biomunitions program included spherical bomblets (M-143), spray tanks (A/B45Y-3), and aerosol bombs (M114 and M33) (4, 20). It is worth mentioning that during the era of the Soviet Union, small bomblets were loaded into Intercontinental Ballistic Missiles (ICBM) like the SS-18, which contained a variety of BW agents and would release five or six BW agents on US population centers during the 1980's (these agents included Anthrax, Smallpox, Plague, and Ebola) (15, 16, 17).

It must be noted that since the early years of the BW offensive program, BW was considered a strategic weapon as it was less expensive than Chemical Warfare (CW) or Nuclear Weapons. For example: to kill the same number of personnel with BW costs about $2 compared to $2000 for chemical weapons, and $2,000,000 for nuclear weapons (4). As techniques for controlling the decay factors of the agents (biological, physical, dispersion) as well as decontamination and prophylactics became better understood, BW became accepted as a tactical weapon as well.

Most applications of BW were used to kill personnel, yet this depended on whether the BW agent had a high or low mortality rate as well as whether the enemy forces were vaccinated or had another means of protection from the BW agent. For example, the selection of a BW agent as an incapacitation agent might be favored over lethality. Incapacitation of forces may be favored in some situations where the affected personnel create a greater drain on the medical and evacuation infrastructure as well as create the expected panic in the general population. Low doses of SEB toxin as well as the diseases Q fever, Venezuelan Equine Encephalitis, and Rift Valley Fever have high morbidity rates (60 to 90 %), but very low mortality rates (less than 1%) and hence would be prime BW candidates for incapacitation goals (2, 4). BW agents with very high mortality rates include Plague (if untreated, pneumonic is 100%), smallpox (30%+ or using the vaccine-resistant strain “ARALSK” 100%), and Anthrax (75%-100%) (4, 17, 18, and 19).

As for BW applications on enemy forces, preparation may be the difference between being combat ready versus losing the battle even before it starts. Just prior to the US led invasion during the Gulf War of 1991, Iraqi forces had been able to cultivate anthrax and had equipped a Mirage jet with a spray tank capable of dispensing lethal anthrax over coalition troops. After the war, the Office of the Secretary of Defense (OSD) commissioned a study of what would have been the potential threat of an anthrax attack on US and coalition forces at the start of ground action. On day one of the ground action, the coalition had assembled half a million military personnel for the attack and 320,000 were assembled along an area of the coastline southeast of Kuwait City. The OSD report states that if the Mirage was ordered to fly by Saddam Hussein and dispersed anthrax upwind from the 320,000 ground forces, then an estimated 76,300 of the 320,000 would have died of anthrax (1). It must be noted that the OSD report also stated that IF all of the 320,000 forces were vaccinated against anthrax, then 122 deaths might have resulted (15).

2. STRATEGIES AND CONSIDERATIONS

A. BW AND CONCEPTS OF WAR

In any war, there are different decision making levels or concepts of the war. The different levels are strategic, operational, and tactical. The Strategic level involves actions and issues of the national interest (e.g. Cold War policy of containment designed by the Pentagon and Joint Chiefs of Staff). The Operational level involves actions and issues of a regional command (e.g. US air and naval bases in Japan as part of Pacific Command). The Tactical level involves actions and issues important to forces engaged on the battlefield (e.g. a column of tanks advancing into Bagdad during Operation Iraqi Freedom or movement of a Special Forces unit on a mountain slope in Kandahar Province, Afghanistan). The Strategic level sets the national policy (given by the Grand Strategic policy set forth by the president in consultation with the Intelligence, Military chiefs, etc.), but it is the Operational level that sets the tactics necessary (and hence provides the planning and resources for the tactics) to achieve the Strategic goals (21, 22, 23, and 24).

BW has been considered in military terms, a "force multiplier" (a tactic or material that can seriously improve one's position on the battlefield) (26). However, BW has differing effects depending on which level of war the BW is applied. Part of the differing effects depend on the above mentioned points: weather, particle dispersal, cost, pathogen mortality rates, number of cells or toxin dosage required to affect enemy personnel, daytime or nighttime application, area of coverage desired, as well as whether the enemy has warning or prophylactic defense against the BW agent. Furthermore, due to the time factor required from exposure to the pathogens to the development of infection which renders the soldier or civilian febrile or unconscious; BW agents may take up to 14 to even 21 days (e.g. Q fever 14 to 21 days; anthrax 1 to 7 days); whereas it is important to note that BW toxins are effective in a shorter time period (Botulism toxin 12- 48 hours, SEB 1 to 6 hours) (4). Thus, chemical warfare agents and nuclear weapons (although more costly to produce and store) are considered more effective against unprotected troops in both a tactical as well as strategic level.

BW becomes useful if the immediate goal does not require immediate death of enemy or vaporization of a city or military installation (25). BW becomes very effective if the goal is to attack strategic or operational centers: industrial cities, military bases, naval ports, government centers, or population centers. BW success would be determined by number of deaths over a short period of time; the taxing and possible collapse of medical and government services; degradation of military readiness; and exhaustion of personnel necessary to maintain national defense or critical national infrastructure. BW would also be effective against troops massing on a border prior to an invasion. It is interesting to note that Soviet Defense Ministry built BW into their military planning to be used not merely for weapons of last resort, but even as conventional (aka nonnuclear) conflicts to attack Strategic and Operational level sites such as population centers, enemy troop reserves, shipping ports, and rail centers (25, 26). In 1989 Ken Alibek, the former first deputy chief of the Biopreparat system (Soviet BW program) was told by a senior military officer that in one attack (some time between 1982 and 1984) by Soviet forces, glanders was used against mujaheddin forces in Afghanistan. The use of glanders (*Burkholderia mallei*) as a BW would have a fatal effect on the mujaheddin transport animals used to manage the mountainous countryside; but it would also lead to fatalities amongst resistance fighters as well- 70% mortality (4). This is a clear example of BW applied as a tactical weapon (16).

Finally, BW has long been considered a tool for use in "asymmetric warfare" (27). Asymmetric warfare is the application of less technological, unconventional weapons, tactics, and strategies. Many times this term had been connected with guerilla warfare, but more recently the term has been used to refer to cyberwarfare and Weapons of Mass Destruction (WMD)-which includes BW! (27). Thus, non-state agents or rogue nations would be attracted to the use of BW to achieve an advantage over other nations, military forces, or civilian targets.

B. BW AS THE "POOR MAN'S A-BOMB"

BW has long been referred to as the "Poor Man's A-Bomb" (15, 25). The advantages for a nation to develop BW weapons are that they are less costly to develop, produce, and store than chemical weapons or nuclear weapons. Many technologies used in the production of making BW agents are the same technologies of fermentation, cell culture, bioprocessing, lyophilization, and milling that are used to make beer, pharmaceuticals, vaccines, and commercial or industrial enzyme products (15, 25). This problem of "Dual-Use" Technologies (i.e. technologies that can be applied for non-military commercial OR military weapons development) makes it difficult to determine if a nation or even an individual is making a BW agent unless actually discovered at the time of BW production. These common production techniques also means that the equipment involved with BW manufacture is very inexpensive (as compared to technologies to develop Chemical Warfare agents or Nuclear weapons). It has been calculated that to obtain the same lethal effect of a nuclear weapon as compared to a BW weapon, one would have to invest $800 for every dollar invested in BW (15).

Also, BW infects damage silently; as opposed to the blast of a nuclear bomb! BW does not need a bomb to disperse the agent. Rather, an aerosol mist of BW agent can be released by airplane crop duster, spray tank on a jet, liquid culture poured into a water source, or even sprayed as a cloud from an off shore ship (note: wind shifts would drive the BW agent inland). Due to the time delay before a disease outbreak is noticed, BW can provide "plausible deniability" to any nation or even terrorist group that chooses to use BW, but then remain silent. Finally, a BW agent could be used to tie up national resources managing the outbreak while the enemy forces then prepare to invade the affected nation; the BW outbreak would hamper military forces trying to stage a counterstrike to the invasion (15).

C. THE CHALLENGES OF BW PROLIFERATION

BW proliferation is the spreading of BW technology, expertise, or even pathogens to other nations or non-state groups (i.e. terrorists). Although many nations are signatories and have ratified the Biological Weapons Convention (BWC) (aka Biological and Toxin Weapons Convention of 1972), many nations are still suspected or have been found to be developing BW. These nations include North Korea, Syria, China, Iran, Libya, Russia, Taiwan, Israel, and Egypt (17, 28).

One challenge to preventing other nations from developing BW technology is controlling “technology transfer”. “Technology Transfer” is the deliberate exchange of technology from one nation to another (or from one nation to a non-state group such as terrorists). Usually the transfer of technology can be through the sale of equipment, blueprints, or even the exchange or emigration of technically knowledgeable personnel who have expertise in that particular technology. After the fall of the Soviet Union, much concern arose over the unemployment of thousands of BW scientists and technicians and whether they would be recruited by other nations to build up their own BW program and expand their BW arsenals. The US provided funding for former BW scientists to develop other commercial products in Russia (e.g. vaccines, pharmaceuticals) as well as other funding to allow former BW scientists to emigrate to the US to work at universities, biotechnology and pharmaceutical firms (27). Unfortunately, other nations such as Iran have hired Russian, Chinese, and North Korean scientists to bolster their BW development (28).

As previously mentioned, one other challenge to BW proliferation is the “Dual Use” technology dilemma. Much of the present day equipment for biotechnology, food fermentation, and pharmaceutical manufacture can be used for the production of commercially valuable products (e.g. beer, vaccines, antibiotics, cytokines, enzymes, etc.) as well as the production of bacteria, viruses, and toxins. Furthermore, the same molecular biology lab equipment used to explore recombinant DNA research could be also used to create genetically engineered pathogens for the next generation BW (27). The problem of “Dual Use” technology is that a perpetrator nation (or terrorist organization) can deny BW development to disarmament inspectors (15). Also, the global availability of this equipment and training for the equipment makes the development of BW inexpensive and the availability of trained personal easy to obtain. Thus, BW can provide even lesser developed nations and/or organizations with limited funds a means to obtain a "level playing field" against Superpower nations with a modest investment in equipment and manpower (15).

D. BW AND TERRORIST APPLICATIONS

Terrorist BW applications has heightened the awareness of BW in the military as well as government resolve to deal with terrorist based BW (aka bioterrorism). Several incidents have fueled military and civilian discussion of biodefense readiness. In April 1990, two attacks by the doomsday cult Aum Shinrikyo, were directed against US personnel based at the Yokohama Naval base in Japan. Both attacks failed, yet due only to the inability of the cult to properly weaponize the botulinum toxin (1, 29). In the fall 2001, the US Senate and other government offices were attacked through the mail with weaponized (i.e. aerosol particles milled to the 1 to 5 micron size for effective intake into the alveoli of the lungs) anthrax spores (29). In 1984, the Bhagwan Shree Rajneesh cult located in Antelope, Oregon, contaminated ten restaurant salad bars with Salmonella bacteria, resulting in at least 750 local citizens sickened (29).

It should be noted that the key motivation for terrorists to obtain and use BW is to inflict causalities, spread terror to the populace, and to weaken the enemy's will to fight (31). Bullock notes seven key characteristics that make BW an ideal weapon for terrorists and rogue nations: ease and low cost of production; ease of dissemination as aerosols; efficient exposure of great numbers of people through inhalation; delayed effect; high potency; high subsequent mortality and morbidity; and, the ability to wreak psychological havoc (30). One other important observation is that terrorists could work with enemy nations to "soften up" the target nation by inflicting a BW attack on a large population or military complex. As causalities start to build up along with quarantines and evacuations, the regional public infrastructure would collapse and the military would be required to maintain order (as well as treat their own BW causalities). Once the combat readiness of the military has been weakened by the domestic BW attack, rendering borders or bases vulnerable; an opposing military could confidently invade with reduced or little resistance (15, 30).

At this point, several issues about bioterrorism effects and the people involved in the attacks arise here. Thomas Glass, a social epidemiologist, raises some important points about the effectiveness of bioterrorism on the society (61). First, Glass notes that the bioterrorist may not get high causality numbers immediately or even high rates of deaths after the event has ended.

Rather, the effect of many bioterrorism attacks will be that the psychological effects will be greater than the physical effects (61). This is especially true if the BW agent is non-communicable (such as anthrax or ricin toxin). Once the BW agent has been neutralized or the site decontaminated (e.g. by sun, chlorine, hazmat personnel), then the danger has passed; but the fear-or terror- generated by the event will still remain.

It is the disruption of our lives and the lingering fear that we do not have a safe society (OR as safe as we once thought) that will magnify the effects of the attack beyond the actual dispersal of BW agent (61). The bioterrorist may not merely wish to inflict causalities or generate fear, but create fear on an economic realm as well. If the BW agent was directed at agricultural resources, then the fear could spread to reduce economic sales and in some cases lead to international bans of exports or imports of agricultural products (61, 62). Finally, Glass via his analysis of emergencies, disasters, and catastrophes, finds that when resources are overwhelmed by the public demand due to a disaster, then first responders must be open to assistance from the public (61). Glass refers to this self-generated public response to assist in disasters as “emergent collective behavior”. In short, the public will rarely panic, but even victims will respond with collective resourcefulness to assist others in need during the disaster. Glass notes that many emergency and disaster rehearsals for BW attacks and other situations usually do not go off as well in real life. Many times, formal response systems break down (example: the communication systems turns out to be the “Achilles Heel”), especially if the disaster overwhelms the first responders and government resources. This is when the public response can be critical to help in the disaster and help reduce causalities (61). Glass’s research suggests that public assistance should not be eschewed by first responders, but cultivated-and well BEFORE the next disaster strikes.

Next, Dick Couch states that there are five principle reasons why terrorists are using weapons of mass destruction (WMD), including obviously BW (2). First, the desire to kill as many people as possible! Second, to exploit the classic weapon of terrorism-fear! Third is the desire to negotiate from a position of unsurpassed strength (ANY credible threat of BW could not be ignored by any sane government and thus the government would be subject to blackmail). Fourth, the terrorists would derive select logistical and psychological advantages due to the delay in signs that the attack has occurred (as in the case of BW, massive numbers of ill and/or dying persons). Remember that many BW agents have an incubation period of hours to weeks before the first symptoms appear. This would offer the bioterrorist the opportunity to escape before the attack is uncovered and to evade apprehension by law enforcement (e.g. the present fall 2001 anthrax attack incident as previously noted would be supported by this point). Also, the failure to capture the bioterrorist and the anonymity during the BW attack would foment further insecurity in the population. Finally, (and as noted previously), the terrorists may want to cause economic or social damage by targeting the national or regional agricultural resources (2). It is interesting to note that Croddy et al (26), cites at the end of his text, that the United States Dept. of Defense believes Osama bin Laden has anthrax and that is part of the reason for the mass vaccination of all military personnel-especially those hunting for bin Laden (26).

Tucker (31) in his assessment of various terrorists and their use of chemical and biological weapons, describe eight attributes of likely BW terrorists. First, there is the tendency to employ even greater levels of violence over time. Many groups start off small, but move to greater weapons and acts of violence as time and their agenda progresses. Second, terrorists are more likely to use BW as they develop innovations in weapons, tactics, and are increasingly more willing to take risks. Third, some psychological studies indicate that individuals with paranoid personalities and a sense of grandiosity are more likely to become terrorists. These traits tend to help the individual disavow any connection with their victims and allow them to devalue and dehumanize potential victims (thereby making it easier to use BW for large scale murder). Fourth, where small groups of terrorists exist, they must consist of a small number of militants. Once the group fragments away from the main group of terrorists, the normative rules active in the larger group are not present; usually it is very small splinter groups that take on more violent actions. Fifth, in some case studies, terrorists with uncertain or undefined constituency or are isolated are more likely to acquire and use BW. Without outside feedback or public support, isolated individuals or groups (as in cults) are more likely to set their own system of morality, be indifferent to adverse public opinion, and thus are more likely to use BW. Sixth, in some groups, a charismatic leader or leadership can influence the group to extreme acts of violence, including BW. Cult-leaders who achieve “god-like” status can manipulate their followers to commit bioterrorism, especially if the leader represents the “supreme standard of morality”. Seventh, terrorists are more likely to use extreme violence if they believe they are in a struggle for survival with an ultimate enemy (or demonized enemy). The enemy can be religious or all-powerful such as right-wing survivalists against the Federal Government. This defensive aggression justifies any violence for the survival of the group and/or self (including BW use). Eighth, some group studies indicate that if the group demonstrates an "apocalyptic ideology" where they are fighting against Satan or other absolute evil to do God's will, then use of BW is possible. The conflict between morals and the use of violence is mollified as the people outside of the religious terrorists are viewed as evil, followers of Satan, unworthy, or otherwise; and therefore, it is morally acceptable to use BW to wipe them out. Under the banner of moral superiority and the grace of a higher power (god), the terrorists may engage in violence for no audience approval, but rather only for themselves and for God to see (31).

Finally, bioterrorism is one aspect of a type of warfare not frequently discussed: asymmetrical warfare. Asymmetrical warfare has been defined by Lt Col Kenneth F. McKenzie, Jr., USMC of the National Defense University as:

"Leveraging inferior tactical or operational strength against American vulnerabilities to achieve disproportionate effect with the aim of undermining American will in order to achieve the asymmetric actor's strategic objectives." (63).

McKenzie's quote was necessary to provide the impact of how bioterrorism overlaps against this definition. With BW, bioterrorists can be a single person or small group and thus they have inferior tactical or operational strength. Bioterrorists leverage their weaknesses in number by using BW aimed at American vulnerabilities (e.g. the general public, unsuspecting restaurant customers as with the Rajneeshees case, someone opening their mail, etc.). Bioterrorists achieve a disproportionate effect by causing deaths; instilling a sense of fear in the public; extensive coverage by the news media; and perhaps a general epidemic-if the BW agent was a communicable disease. Also, other aspects of this disproportionate effect could include-depending on the BW agent and Bioterrorist's target: loss of agricultural crops, drop in economic activity (for food products, agricultural trade, lumber if infected with anti-crop pathogens), drop in the stock market, reduction of consumer activity in malls or sports stadium (if the last bioterrorism attack occurred in a shopping mall or sports stadium). The social chaos, psychological fear, and economic effects of a BW attack are the means by which the bioterrorist has undermined the will of the American people.

Kolodzie accurately describes asymmetrical warfare as attacks best used against targets with little or no protection (64). This description fits well with the bioterrorist's targets. Kolodzie further suggests that to prepare for this type of warfare-which has been defined by some as similar or synonymous with terrorism (64)-one must constantly be training for this occurrence. In essence, plan to think the unthinkable.

3. BRIEF HISTORICAL EXAMPLES-SEE TABLE 1

As previously demonstrated, the application of BW in military conflicts goes back centuries. In 1346, during the siege of Kaffa (now Feodosia on the Crimean coast), Mongols catapulted plague infected cadavers into the fortified city and plague spread throughout the city. It is easy to comprehend the outbreak since plague bacteria can enter cuts and abrasions on the hands, while city dwellers removed the cadavers and/or body fluids from the city streets for example. Even though the Mongols did not understand Germ Theory, they understood the concept of contagion. Hence, using the contagion from the cadavers to achieve the spread of plague inside the city, the Mongols achieved the fall of Kaffa and the evacuation of its Genoese merchants (32).

Smallpox was another disease used in early forms of BW in the 1700's. In 1763, during the Pontiac Rebellion (aka Indian Wars), the British were struggling to maintain their major outpost at Fort Pitt, (located in western Pennsylvania) under siege by Indian attacks (32). Although various historical accounts are conflicting, Sir Jeffrey Amherst gave the orders to Colonel Henry Bouquet (who was heading to Fort Pitt with reinforcements) to infect smallpox on the Indian population using infected blankets. Bouquet upon arrival at the fort, gave two blankets and a handkerchief to hostile chiefs (14). Although various scholars debate whether the infected cloths contributed to the spread of smallpox (14, 32), it was known that the pus exudates of smallpox can contain active smallpox virus and hence spread the disease. Furthermore, Native American Indians were very susceptible to smallpox and mortality rates were high as smallpox was a disease imported to North America via the European settlers (33). Within months after Bouquet's arrival, the siege of Fort Pitt ended.

During the American Revolutionary War, the use of smallpox was a powerful BW tool. British troops were vaccinated (via variolation), whereas American colonists were not. During the siege of Boston to remove encamped British forces, smallpox broke out in the city in

December 1775 (33). British General William Howe ordered all British troops variolated and then variolated civilian refugees in hopes of spreading the disease to susceptible Colonial forces outside of the city. General Washington upon hearing the news of the smallpox epidemic, delayed the liberation of Boston, and unfortunately his troops suffered from the smallpox outbreak (33).

During the siege of Quebec City in December 1775, Continental forces under Benedict Arnold were poised to seize the fortress city. The British fort commander had civilians variolated and then had them mingle with Continental troops. With a few weeks, a severe smallpox epidemic broke out affecting nearly half of the ten thousand Continental troops. After burying the dead, The American forces retreated in disarray to Fort Ticonderoga (32, 33). As a result, of repeated smallpox outbreaks amongst Colonial troops, General George Washington-himself scarred by smallpox at nineteen, ordered in 1777 the entire Continental army variolated before he launched any new military operations (33).

During World War I (WWI), Germany's military command thought it was barbaric to use BW against soldiers, yet conducted an active campaign of BW using anthrax and glanders to infect military draft, cavalry, and livestock (e.g. horses, mules, cattle) (14, 34, and 35). These BW attacks occurred in Bucharest and Mesopotamia as well as within neutral nations supplying military animal stocks to Allied forces (e.g. US). One of the best known BW agents was the American-born, German heritage physician, Anton Dilger. Dilger recruited by the German military command, worked out his of Chevy Chase, MD basement producing BW cultures of Glanders and Anthrax which were used to infect mules and horses intended for export to Allied forces in Europe (34, 35).

By the time WWII began, many countries began or had active BW programs. Although Adolph Hitler was against using offensive BW for fear of Allied retaliation using their own BW, Nazi Germany had a limited BW offensive program in development, but never used any of their products. It is interesting to note that during WWII, Germany produced defensive BW such as vaccines and sera against plague weapons from the Soviet Union as well as a variety of anti-crop and anti-animal BW weapons including potato beetles, blight, choking weeds, and Foot-and-Mouth Disease (36). During WWII, Britain, US, Canada, and all had active BW offensive programs and shared information and research amongst each others programs, but no offensive BW was used against any of the Axis Powers (14, 26). The USSR BW program was active and the Soviets were believed to have used unsuccessfully Tularemia against the German invasion (4, 16). As previously mentioned, Japan was one of the few nations during WWII to have an active BW program and to have used BW against Chinese civilians and tested BW weapons against live prisoners (10).

4. BW TREATIES

Before WWI, a variety of international agreements and codes of conduct existed that prohibited the use of poisons or so-called “poisoned weapons”. These include the Strasbourg Agreement between France and Germany (1675), the Lieber Code of the US Army (1863), and the Hague Peace Conventions of 1899 and 1907 (these last two included prohibitions against the use of infected carcasses to poison wells (37). Yet, even the Greeks and Romans condemned the use of poison weapons in war as a violation of the “law of nations” and around 500 BC, the Manu Law of India forbid poison weapons considering them “inhumane” (38).

Yet, after WWI and the extensive use of Chemical Weapons, the League of Nations began a process to ban chemical weapons. After several commissions were assembled to debate and review the issues of chemical and bacteriological disarmament, it was the Polish delegates to the League of Nations that proposed and promoted inclusion of bacteriological weapons in the treaty that came to be known as the “1925 Geneva Conference” (later called the Geneva Protocol). The most noted speech on inclusion of banning bacteriological weapons in the Conference was General Kazimierz Sosnkowski, who explained that bacteriological weapons could be easily hidden, could easily lead to mass extermination of “men, animals, and plants”, as well as easily lead to epidemics (37). Although between 1925 and 1939 most of the major powers ratified the Geneva Protocol, the US and Japan did not (37). Some nations did not consider BW was a viable tool of warfare (26). Also, the USSR and Japan continued extensive offensive BW research and preparations during this period (26).

After WWII, the arms race between the US and Soviet Union expanded beyond nuclear and chemical weapons and also included extensive research and development of BW-both pathogenic agents as well as manufacturing and delivery systems (16, 39).

On November 25, 1969, US President Richard Nixon surprised the world by announcing the unilateral abolition of BW (39). Privately, in response to inquiries by the Secretary of Defense Melvin Laird, Nixon told him that any nation that used BW on the US, “we’ll nuke ‘em” (39). Within one year, the Soviet Union accepted the opportunity to develop a BW disarmament treaty. The Biological and Toxin Weapons Convention (BWC), signed in April 1972, was the first multilateral treaty in recent history to ban an entire category of weapons. The BWC was ratified by many major powers (including the US, Great Britain, France, and USSR) and went into effect in 1975. The treaty states that all biological weapons and delivery systems are to be destroyed and only defense research is permitted. Also, any nation can report to the United States of another nation that is cheating on the BWC. Finally, one provision of the treaty requires member states to assemble for a review conference (also called Confidence Building Measures) to review the status of the treaty and work on any scientific or technological developments that may have arisen (39, 40, and 67).

Unfortunately, even in 1975, nations began to cheat on the BWC treaty. The most notable violators to the BWC are the Soviet Union (Biopreparat program), South Africa (Project Coast) (41), North Korea, and Iraq (16, 38, 39, 41). Shoham and Wolfson (17) present evidence in 2004, that despite Russian assurances and the BW declaration by former President Yeltsin in 1992, the Russian military is still a threat as it still possesses stockpiles of BW as well as BW production capabilities (25). Furthermore, despite BWC confidence building measures and discussions on trying to prevent terrorists from obtaining BW (40, 42), there are at present greater concerns that rogue nations or terrorists will obtain BW technology and use it. These concerns are fueled by CIA evidence of Russia assistance to North Korea in the development of advanced anthrax BW, including ultraviolet (UV) light resistant forms (39); Russian scientific assistance to Iran in the development of biotechnology and BW (26, 43); Al Qaeda’s interest and efforts in development of BW and other WMD (13, 44). Evidence of Al Qaeda’s BW interest includes BW production documents secured from training facilities in Afghanistan (13) as well as a senior bin Laden associate who in 1999 while on trail in Egypt, declared that Al Qaeda had BW as well as chemical weapons (2).

5. PRESENT POLICIES AND METHODS OF DEFENSE AND DETERRENCE

Ainscough presents one remarkable quote: “the First World War was chemical; the Second World War was nuclear; and that the third World War-God forbid-will be biological.” (27).

Davis (29) presents a wake up call by attacking the prevalent myths of BW. These six myths are: absence of a significant BW attack; the US has never been attacked by a BW agent; BW requires very intelligent, very educated, and highly funded program to produce, weaponize, and employ a BW agent; BW must be too difficult as previous attempts have failed; there are moral restraints that have kept BW from being used; and the long incubation period required for BW make it useless to users. Many of these arguments have been dashed to the ground by the September 2001 attack on the US by Al Qaeda (e.g. flying airplanes into buildings) and the subsequent anthrax attack via mailings to targeted Senators and others. We must confront the reality that there are some groups or individuals willing to die to achieve their goal of terror on an innocent population. Yet, history will remind us that the Aum Shinrikyo and Bhagwan Shree Rajneesh used BW in their attacks. Furthermore, the Germans used BW against animals in WWI, the Japanese used BW in WWII, and the British used BW in 1763 and during the American Revolutionary War. Also, we can not forget the strategy of poisoning drinking water using dead animals in the US Civil War or farther back to the time of the Greeks and Romans. Finally, we are compelled to recognize that any treaty (e.g. Geneva Protocol of 1925 or BWC) is merely ink on paper to those who choose to cheat on any treaty (e.g. USSR, Iraq, South Africa) or are non-state entities and never agree to a treaty (Al Qaeda or Aum Shinrikyo). Therefore, we must confront strategies of BW defense and deterrence.

Deterrence against BW is difficult to understand unless one considers what would be the response by the government subject to the BW attack and now dealing with personnel ill or dead due to the BW attack. To return to the "nuke 'em" quote by President Richard Nixon, Lebeda discusses this deterrence strategy in light of diplomatic exchanges between the US and Iraq prior to the Gulf War of 1991 (aka Operation Desert Storm). President George H. W. Bush and other high level US officials made it clear that a severe response would occur if Iraq used BW (or Chemical Weapons) against Coalition forces (49). Iraqi officials read this to be a form of "escalatory deterrence" (i.e. use of nuclear weapons) (49). Lebeda notes that "to deter" means to convince the enemy that the cost of aggression exceeds any possible gain. The author discusses this concept in a variety of actions: Military, Diplomatic, and Defensive. Briefly, the deterrence by military action always leaves the US with the option of retaliation using nuclear weapons. Also, Lebeda notes that "Retaliation in kind", such as the US retaliating with chemical or biological weapons tends to confuse the political arena and confound efforts to develop new international treaties to control WMD proliferation and production. Deterrence by diplomatic action returns to the BWC and Geneva Protocol of 1925 to maintain political and diplomatic pressure against nations possessing BW.

Also, in the diplomatic arena, inspections and verification efforts can be required with which the results are reported to the United Nations. Deterrence by defensive action serves as the final leg of the triad that builds up a web of deterrence. Defensive action plays a key role in developing medical (drugs, antibiotics, vaccines, diagnostic tests) and nonmedical countermeasures (masks, detectors, protective over garments, and shelters) which deny the adversary the maximum benefit from BW. In essence, if the BW agent will not create the high number of casualties, then it is not worth the expense to develop; not worth the chance it might return back and create a local epidemic; and not worth the chance that the enemy will retaliate with more powerful weapons (49).

Furthermore, the defense against any BW attack must include Human Intelligence (HUMINT) as BW can be made or transported in small facilities or hidden from spy satellite view via underground facilities (43). It is the intelligence agent observing the bioreactor making anthrax, or the BW filled SCUD, or the dispersal of BW into a reservoir or a water tank that can make the difference in locating and connecting the BW attack with the perpetrators. Thus, the knowledge to connect the adversary making, storing, or releasing BW may require improvements in HUMINT.

In a situation of BW release, whether on a battlefield or a bioterrorist attack on civilians, one level of protection for military personnel that is essential is the means to prevent inhalation of the BW agent or prevent the agent of coming in contact with skin, mucous membranes, or the eyes. This is especially important for toxins such as T2 mycotoxins which can be absorbed by skin, whereas skin abrasions can permit the entry of various pathogens like anthrax, plague, smallpox, or Ebola. During the history of the US BW program, various masks were developed. The present day mask, the M40, has a molded silicone face piece, with a voice mitter, drinking tube, as well as filter canister mounts (2, 4). The mask filter (US C-2 canister) acts to filter particles from the air via an activated carbon filter as well as an electrostatic filter. Also, other protective garments include: battle dress over garment (used once for a 24 hour period and upon contamination, it is burned), chemical protective undergarment (used for protection against chemical warfare agents), vinyl rubber over boots (worn over the standard military boot) and vinyl rubber gloves. The Military Operations Protective Posture (MOPP) conditions are levels under which part of the protective garments or all garments are worn-depending on the hot weather or type of agent present. For example: MOPP-4, all garments and mask are worn; MOPP-0 all garments and mask carried or available, but not worn (2, 4).

After the agent has been released, detection of the agent is necessary, especially since signs of illness may take up to 14 days after the initial exposure to the BW agent. A variety of monitoring and detection devices exist in the US military. The Long Range Biological Stand-off Detector System (LRBSDS) uses an infrared laser to provide an early warning of an aerosol cloud at a distance of up to 18 miles. The Biological Integrated Detection System (BIDS) is a vehicle mounted portable lab that samples aerosol particles and subjects them to a variety of genetic and antibody-based tests to identify the BW agent present. Both devises are being fielded by the military and can be adapted for first-responder civilian applications (2).

Recent advances in nucleic acid chemistry and genetic amplification technology has heralded more extensive and quicker tests for a variety of BW agents. Many are based on Polymerase Chain Reaction (PCR) techniques as well as DNA binding probes with fluorescent dyes that bind to specific sequences of the unique BW agents (45). Yet, many of these diagnostics must withstand the demands of field use: that is, must be portable, rugged, and rapid in detection and analysis. The Ruggedized Advanced Pathogen Identification Device (RAPID) has the advances of being able to withstand field requirements and at the same time uses a composite capillary tube to provide two forms of pathogen detection protocols: screen tests to analyze samples for the presence of multiple different organisms simultaneously, AND provide batch tests to analyze multiple samples for a single organism (45). Henchal et al (46) notes that since no one test will be able to identify all BW agents, a diagnostic system that combines clinical diagnosis and medical intelligence with immunodiagnostic tests, rapid gene amplification assays (e.g. RAPID), and standard microbiological tests (e.g. microbial cultures) will provide results with the highest quality and greatest confidence in BW detection and diagnostics. If these techniques are distributed throughout a network of military and civilian laboratories, then rapid detection of BW agents after a BW attack would be assured as will the rapid treatment of BW victims (46).

Culpepper and Pratt (47) note that advances in medical BW defense will require improvements in vaccine technology to provide more immunization defenses against BW agents.

These advances will be required to cover several pathogens at the same vaccination as well as provide defense against genetically engineered BW agents (more on BLACK BIOLOGY below). Although there is some success with the Anthrax and Smallpox vaccine, other BW pathogens (e.g. Ebola, Ricin, or Brucellosis) do not at present exist (5, 48). These next generation vaccines will be constructed using Naked DNA, Chimeric Antigens, Synthetic Peptide based, or RNA replicons from alpha virus platforms (47). The authors state that the defense research program will create conditions where the aggressor might be less inclined to use the BW agent in the battlefield; especially since most of the military forces are immune to the BW pathogen (47).

Although an array of antibiotic drugs exist today, only some select ones are effective for a specific BW agent (e.g. doxycycline or ciprofloxacin for anthrax). The challenge to BW antibiotic therapeutics is developing antibiotics or antivirals that are effective against BW agents. This will be further complicated by genetically engineered BW agents that are resistant to many antibiotics (3-more on Black Biology below). Alibek suggested the development of non-specific immune modulators that would stimulate the innate immune defenses for any BW agent (16). Some progress was reported by Cerys Rees et al, using synthetic cytosine and guanosine (CpG) DNA as a generic therapy against infectious diseases (50). Although this research is early in the program, it is hoped that various CpG sequences can promote and/or activate macrophages, monocytes, dendritic cells, NK cells, and the complement system to overcome various BW agents (50).

Finally, BW defense specialists must have a criterion to differentiate whether an infectious disease is a natural epidemic or a BW attack. Noah et al (51) discusses the epidemiological distinctions by focusing on a questionnaire that examines the epidemiological data of the disease, location of the outbreak, temporal patterns of the disease, number of cases, unusual strain or variant of the pathogen, morbidity/mortality rates, previous history of an endemic outbreak of the disease, seasonal distribution, antimicrobial resistance patterns, zoonotic potential, as well as the proportion of combatants among the population at risk. In examining these factors, a BW trained physician can make a determination whether the disease outbreak heralds from a BW attack or a natural epidemic (51).

Also, since many first symptoms of BW agents are similar, how do physicians differentiate the illnesses to speed up treatment and reduce patient mortality? Wiener (52) describes a strategy of asking questions to determine the BW agent, the analysis of symptoms to identify the specific pathogen against other possible pathogens, what personnel are vulnerable, as well as what countermeasures are possible. Again, this is part of BW deterrence since the means to quickly ascertain whether a BW attack has occurs (versus a natural outbreak of an unusual pathogen), diagnose the best possible treatment and determine what other countermeasures (e.g. masks, MOPP-4, vaccination, antibiotics) are necessary to prevent further morbidity or mortality (52).

6. PRESENT THREAT

Pravecek and Davis paraphrase a threat determination formula devised by Lt. Col. Don Noah, USAF (53). The threat of BW can be quantified by integrating the following variables: An adversary's *intent* to use BW; an adversary's *capability* to use BW; our own *vulnerability* to BW.

In essence:

Enemy Intent + Enemy Capability + US/Allied Vulnerability = Threat (53)

The present threat is very real as the US is still slow in the development of the necessary vaccines and drugs to deal with some BW agents (5, 38). Furthermore, even with treaties meant to stop BW weapons development, nations have been cheating and other nations or terrorists have been trying to develop BW (2, 13, 16, 38, 39, and 41). Also, the problem of technology transfer arises, when the same technology used to make vaccines or drugs can be used to cultivate BW agents. Finally, due to the dilemma of "dual-use" technology, the equipment to make BW agents is inexpensive and openly available on the global marketplace. Kathleen C. Bailey, former assistant director of the US Arms Control and Disarmament Agency (USCDA), stated that she is convinced that a major biological arsenal could be built with $10,000 worth of biotechnology equipment in a room 15 feet by 15 feet (38). Finally, the application of BW agents as aerosols is quite easy and can require a modern spray unit attached to any airplane. Al Qaeda operatives generated a lot of fear when it was reported in the news that the operatives had explored renting crop dusting aircraft (29). The news media reported that a BW attack might occur.

But beyond aerosol attacks, BW attacks can come from other directions. Hickman (54) examined the vulnerability of water systems (both military and civilian) to BW attack. In his analysis, he identifies critical points which if left vulnerable, could be targets for BW or chemical weapons (CW) and thus render USAF operations-which depend on that water-neutralized or dead. Hickman proposes several steps to improve force protection of these critical water supplies, including: focus on water system vulnerability assessment, review of Civil Engineering water system outsourcing and management practices, and re-evaluation of the CW and BW conventional wisdom of threat and risks (54). Hickman is not unusual in examining the BW threat to water systems. Project Coast in South Africa used the BW agent cholera in river water supplies to attack forces opposed to white South African rule (39).

Furthermore, BW has been developed against agricultural targets, both crops and animals. The threat to a nation's economy would be great if BW was directed at the agricultural products that a nation produces (12). According to Wilson et al, potential targets of agricultural BW would include: farm animals (including livestock, poultry, and fish), field crops (including grains, trees, fruits, and vegetables), processed food, and agricultural storage facilities. During the 20th Century a variety of plant pathogens were developed for agricultural BW, including ergot, wheat rust, rice rust, and potato blight. But Wilson also describes how various animal based agricultural BW agents including glanders and anthrax were used with varying success in the 20th Century. Wilson finally warns that four key pathogens are prime for BW attacks against agriculture. These include: Foot-and-Mouth Disease, Avian Influenza, Classic Swine Fever (aka Hog Cholera), and Newcastle Disease. Wilson concludes with a call for vigilance using an intelligence and surveillance system that is responsive to animal disease and the needs of agriculture (12).

Peterson (13) discusses the problems with agroterrorism using Foot-and-Mouth Disease (FMD). If FMD was distributed in the US by terrorists as a BW agent, Peterson states that Americans could expect an immediate and sustained increase in the price of food as well as an economic catastrophe due to need to destroy vast numbers of infected cattle (13). Peterson's recommendations include getting lawmakers to change the definition of "Weapons of Mass Destruction", (title 50, chapter 40) in the US Code on the Defense against Weapons of Mass Destruction Act to include agricultural diseases, not merely just human diseases. Furthermore, Peterson recommends that the US Department of Agriculture (USDA) build up their infrastructure with state agricultural agencies and that the Department of Homeland Security (DHS) and the Department of Defense (DoD) provide help to the USDA Animal and Plant Health Inspection Service (APHIS) to deal with future agroterrorism attacks (13).

7. FUTURE THREATS DUE TO TECHNOLOGICAL DEVELOPMENTS

A. BIOCRUISE DELIVERY

With the development of new technologies such as cruise missiles, new threats involving BW will arise on the horizon. Biocruise is defined as the combining of BW technology with cruise missile delivery systems. A cruise missile is defined as "an unmanned self-propelled guided vehicle that sustains flight through aerodynamic life for most of its flight path and whose primary mission is to place an ordnance or special payload on a target."(55). This definition today includes unmanned air vehicles (UAVs) and remotely piloted helicopters or aircraft (RPVs). Cruise missiles are easier to obtain, maintain, weaponize, and employ than ballistic missiles. Ballistic missiles are not favored as BW delivery vehicles due to the speed and heat generated during re-entry on the warhead or nosecone of the missile. Since many BW agents can be destroyed by the heat and blast effects from a warhead, spraying as an aerosol is the most favored method of dispersal for a BW agent. Cruise missiles have the advantage that a properly sized aerosol dispersal system could be installed within the missile. Once installed, the cruise missile could deliver a BW aerosol over a large swath onto a densely populated area resulting in mass causalities (55). Some cruise missiles have extremely accurate navigation systems, using terrain contour matching (TERCOM) guidance systems, whereas others have guidance systems using the Russian Global Navigation Satellite System (GLONASS), US Global Positioning System (GPS) or the Differential GPS (DGPS) systems. With these systems, the accuracy of targeting by cruise missiles is far superior to ballistic missiles (55).

Kiziah (28) discusses the biocruise threat from the perspective that a biocruise attack could provide "plausible deniability" from a rogue nation. If the attack was done at night, a long range land attack cruise missile (LACM) could be directed to disperse the BW agent while programmed to fly a circuitous route to the target. After dispersal, the missile could be programmed to crash in the ocean or self destruct. Since cruise missiles fly low, (some below radar detection level) as well as have a small Infrared (IR) and radar signature; this makes detection of cruise missiles difficult. Further, it must be noted that cruise missiles can be launched from sea (even launched covertly from a cargo or tanker ship), from the air, as well as from a submarine. Kiziah (28) also discusses the problem of cruise missile proliferation, especially to rogue states. The major proliferation pathways for rogue nations are: direct purchase of complete LACM from another country; indigenous development of LACMs; with or without outside assistance; and conversion of anti-ship cruise missiles or UAVs to LACMs. The proliferation of cruise missile technology will only enhance the threat of biocruise and a serious BW attack on civilian or military centers in the coming decades (28). Also, as rogue nations may transfer technology and weapons to terrorist groups, it is conceivable that the threat from a terrorist based biocruise will increase or in fact occur.

B. BLACK BIOLOGY

Black Biology is defined as the use of recombinant DNA technology towards the development of Biological Weapons (68). With the rise of biotechnology and the understanding of molecular biology of pathogens, the applications of recombinant DNA to enhance the virulence of BW agents began (16, 27). One example of black biology was the work done by Sergei Popov, a department chief in the Soviet bioweapons program (16, 27, and 43). Popov was able to insert the myelin-producing gene into Legionella. Upon infection of guinea pigs, the pathogen created a delayed neurological degeneration syndrome; the guinea pig immune system eventually destroyed the myelin sheaths on the guinea pig nerves resulting in paralysis. Popov also reported success in developing a strain of plague that was resistant to multiple antibiotics and a strain of anthrax that was resistant to both the anthrax vaccine and multiple antibiotics.

Ainscough (27) describes the revolution in biotechnology and molecular biology as a potential Revolution in Military Affairs (RMAs). RMAs require four essential elements: technological advancement, incorporation of this new technology into military systems, military operational innovation, and organizational adaptation that basically alters the character and conduct of the conflict. With the onset of genetic engineering, it is only a matter of time before black biology creates new BW that become the RMAs of the 21st Century (27). In 1997, a group of academic scientists (the JASONS Group) met to discuss the threat of black biology on BW. This group of scientists defined six broad groups of genetically engineered future threats: Binary

BW (two part innocuous system that become lethal once mixed together); Designer genes (genes added to pathogens to create new combinations of diseases); Gene therapy as a weapons (using the techniques of gene insertion to deliver sickness or death into the cells) (69); Stealth viruses (using a cryptic viral infection to deliver death at a latter time or to a targeted segment of the population); Host-swapping diseases (creating diseases such as a virus that switches species targets and hence would be highly virulent to the new host); and finally designer diseases (diseases created to target a desired set of tissues and create a desired set of symptoms) (27).

Daly (3) replies to the threat of black biology by examining how understanding the genomics of various organisms will help in understanding genetically altered BW. Daly discusses how some extremophiles and their genes for specific traits could be used for genetically enhanced BW. For example, using thermophiles (heat loving organisms) might provide traits to build better heat resistant BW that would withstand explosive dispersal from a missile or withstand the febrile state inside of human hosts. Barophiles (pressure loving organisms) traits could help design BW that withstands the high pressures during the detonation of a BW warhead. Radiation resistant bacteria, such as *Deinococcus radiodurans*, could provide traits to create BW that is resistant to radiation as well as desiccation (3). Daly states that *D. radiodurans* would be a good candidate for the development into a BW agent because of the following qualities: extreme resistance to acute and chronic radiation; extreme resistance to desiccation; high resistance to decontamination via disinfectants; very tolerant to solvents; and highly amenable to genetic engineering. Daly notes that in the future, rather than build a BW organism from scratch, it may be simpler to engineer BW attributes from 1 up to 4 traits into organisms that are naturally environmentally robust.

Finally, Zilinskas (56) discusses some of the targeted traits that black biology could be directed to improve BW agents. These include increased hardiness against desiccation or UV damage; resistance to antibiotics or antiviral drugs; enhanced infectiousness by enhanced binding to target cells; increased pathogenicity by enhancing virulence factors (e.g. local effect enzymes, distant effects toxins, and evasion of host defenses); modification of host specificity (either expanding the host targets of non-human pathogens to humans or reducing the host specificity to select ethnic or racial groups); increased detection avoidance (altering antigens of a pathogen so the immunized patient still becomes infected); and modified senescence (cells self-destruct on cue). Zilinskas describes one very important point at the conclusion of his paper. Zilinskas (56) states that he has not seen much discussion on the problem of pleiotropic effects for genetically altered BW agents. Pleiotropic effects are the genetic effects of one gene on multiple traits. It can also be described as the unforeseen effects due to the genetic manipulation of the organism. For example, by inserting other genes into an organism, the organism may not act as robustly, but rather become very fragile and unstable in many environments. These pleiotropic effects may result in an undesirable BW agent. Antagonistic pleiotropy refers to the expression of a gene that causes multiple competing effects (some maybe beneficial, whereas others maybe be detrimental). BUT, if the genetically altered BW agent does not undergo "field testing" before use, it could be possible that the pleiotropic effects might result in more damage to the environment or result in an uncontrollable epidemic (56).

8. BIODEFENSE EFFORTS AND RECOMMENDATIONS

A. CHALLENGES

The challenges to the military dealing with BW will be to "think the unthinkable" (53). In the coming years, more advances in detection technology will be directed at early detection ("detect to warn" rather than "detect to treat"), rather than detection after the BW agent is present and personnel are demonstrating symptoms. This will be difficult as the nature of BW is to allow for clandestine dispersal and the only evidence may appear in emergency rooms and doctor's offices (30).

Treatments will need further advancement including research on any new genetically modified BW agents that arise. Alibek and Cerys Rees's work (16, 50) will be critical to create non-specific immune responds that will allow for a blanket immune response to any BW agent. Nonspecific immune responses may also be the critical first line of defense during a BW attack with a genetically altered agent. Some defense the agent would be better than no treatment at all.

Containment of dual use technologies and technology transfer of BW will be difficult. This will eventually require coordinated exchange of information amongst various intelligence and law enforcement agencies across the globe. Although funding to re-direct BW scientists into more productive work (such as what occurred in Russia in the 1990's) has borne some fruit; many other scientists and technicians have been reported hired in other countries, perhaps sharing designs of old Soviet BW technology (43).

Communication needs to be enhanced between various government agencies (Defense, Homeland Security, Agriculture, Centers for Disease Control) as well as local and state agencies and testing labs. Much of the information can be electronically transferred. In the age of the Internet, the Program for Monitoring Emerging Diseases (ProMED), provides communication with sentinel stations across the globe that report unusual disease outbreaks (30). It is conceivable that with the global membrane of communication-the Internet-communication between the testing labs, the first responders, the agency directors, the law enforcement, the military, and the intelligence agencies can be woven together into a real time instant meeting sharing data. Planning strategies, issuing evacuations and quarantines, and distribution of vaccine or drug treatments to the population could be rapidly and effectively coordinated. By effective use of information technology, it is possible to reduce the time from the actual BW attack to treatment which would reduce the mortality rates and hinder the success of the BW attack.

Finally, in deterrence, governments (globally, not just the US) must make it very clear the consequences of a BW attack by an aggressor nation, rogue state, or terrorist organization (49). Any nation that uses the deterrence of punishment or escalatory acts, must not only have the capability to issue the deterrence, but the will to issue the action if the BW event occurs (49).

B. DRILLS

One other means to deal with the biodefense challenge is simulated drills of BW attacks. Drills provide administrators and key personnel with concepts and experience in hopes that they will improve their work performance during an actual BW event. TOPOFF (name stems from the drill engaging only “Top Officials” of the US Government) was a $3 million exercise which took place in May 2000 (57). Its purpose was to test the readiness of top government officials to respond to terrorist attacks directed at multiple geographic locations. In three US cities the following events took place: Portsmouth, NH a chemical weapons event; in Washington, DC a radiological event; and in Denver, CO a bioweapons event. TOPOFF was intended to be “player driven” event (i.e. the participants decisions and the subsequent consequences were the primary drivers in the shaping of the exercise). TOPOFF was also a “no notice” drill (i.e. participants were given no formal advanced notice of the nature or timing of the event so their reactions and decisions were as close to reality as possible).

Inglesby (57) describes the results of the TOPOFF drill. The drill revealed problems with leadership and decision-making as well as difficulties of priorities and the distribution of scare resources. TOPOFF also revealed the discord created as contagious epidemics strain health care facilities as well as the need to develop sound principles of disease containment. Flaws in the distribution of drugs to the population were also revealed by this drill. Overall, TOPOFF provided lessons that would help shape future bioterrorism response planning at all levels of government (57).

Other tools to assist in drill simulations include scenarios designed by BW and public health specialists. The reader of the scenario can analyze what critical component or resource is necessary to control or contain the BW attack. O'Toole (58) uses a bioterrorist attack using smallpox to demonstrate how failures in communication can lead to an initial attack and subsequent failures to contain the outbreak which later blossoms into an epidemic. O'Toole further describes how fear to enact and enforce quarantine-for the hospital and for the city leads to further spread of the disease. In short, the spread of the smallpox, could be averted by training medical personnel in identifying smallpox (even if they have not seen a case in decades), educating the public on the need for quarantines (even in the 21st Century), making local government more responsive to use emergency powers and enforce quarantines, and speed up the distribution of vaccines to contain or prevent further spread of smallpox (58).

C. BIODEFENSE 85%

Finally, Biodefense Now 85% (Biodefense) was a project to attempt to determine if there were any quick-to-implement ideas using available technologies or capabilities to enhance the protection of military forces against BW (53). The premise was that the 100% defense solution was difficult, if not impossible, to obtain. The ultimate goal was to reduce the BW threat to US and allied forces at fixed bases. The goal was to focus on improving protection in a short time frame of two years (by 2006).

The workshop for Biodefense occurred on October 20, 2004 in Washington, DC where forty-one workshop attendees were divided into four groups. The attendees came from various military, academic, and industrial firms with experience, knowledge, or skills dealing with BW. The groups generated 56 ideas directed at providing a substantial amount of additional protection against BW attack. In order to filter down this number of ideas to a more management number for the Department of Defense (DoD) to act on, the workshop attendees reviewed all of the 56 ideas and ranked their top 15 choices into three categories (Implemented Quickest, Greatest Benefit, and Implemented Quickest and Greatest Benefit). In reviewing the results, the recommendations ranged from vaccinate all personnel, to educational training for select or all personnel, to modifications of the ventilation systems for base facilities (53).

It is interesting to note that the idea voted Best Overall as well as Greatest Benefit was called C-BW CONOPS. This term Counter-Biological Warfare Concept of Operations (C-BW CONOPS) means that a doctrine developed by the Combatant Commanders and services, should be developed for all military operations in a BW contaminated environment. This doctrine of operations should be comprehensive for all personnel and for all operations and should address issues such as airfield operations, deployment and redeployment of forces, cargo transport, operating in contaminated areas, re-supply, and disposition of BW-contaminated remains and mass causalities. The authors emphasized that BW should not be sequestered into merely medical or disaster planning, but should be an integral part of war plans, operations, and training. The idea voted Implemented Quickest was the Weekly Commander's Stand-up Briefings. These briefings would provide uniform and frequent briefing to base commanders regarding illness trends. At these briefings, overall disease trends should be reported, including occurrences of infectious diseases such as the flu. The briefing may change to more frequently depending on the threat level (53).

Overall, this process brought together key parts of rapid problem solving, decision making with a team of BW experts, and provided information that DoD could rapidly implement for improving Military facilities readiness to handle BW attacks. It must also be noted that BOTH top ideas focused on information and communication within the military structure.

9. CONCLUSIONS

In conclusion, the development of BW proliferation and genetically altered BW agents as well as new technologies such as cruise missiles and the dual use technologies will continue to challenge military readiness in the face of BW attack. These challenges will demand improvements in communication, detection, treatment, and prevention as the military deals with BW threats from the battlefield and from terrorist attacks. Furthermore, since the military will be called to assist civilian needs during a bioterrorist attack, it will require the military to enhance their communication and detection resources to better serve civilian needs. With the global threats by rogue nations and terrorists as well as groups capable of obtaining BW technology, it is conceivable that the 21^{st} century WILL be the century of BW.

TABLE 1-TIME LINE OF IMPORTANT CONCEPTS FOR THE DEVELOPMENT OF BIOLOGICAL WEAPONS		
TIME PERIOD	CONCEPT	EXAMPLE
PREHISTORIC TO 1800'S	CONCEPT OF CONTAGION	-1770 BC-SUMERIAN CUNEIFORM TABLETS, ARCHIVES OF MARI, ANCIENT SUMER (SYRIA), FIRST RECORD OF "CONTAGION CONCEPT". -CONTAMINATION OF ENEMY WELLS WITH CORPSES BY ROMANS, GREEKS, AND OTHER ARMIES 1763- BRITISH GENERAL AMHERST'S USE OF SMALLPOX CONTAMINATED BLANKETS TO AMERICAN INDIANS
1860-1880	MIASMA THEORY VERSUS GERM THEORY	-PASTEUR'S WORK -KOCH'S POSTULATE -BACTERIAL PATHOGEN IDENTIFICATION BY VARIOUS MICROBIOLOGISTS
1900 TO 1920'S	EARLY "MODERN" BIOLOGICAL WEAPONS-ANIMALS ONLY	-GERMAN AGENT ANTON DILGER'S USE OF ANTHRAX AGAINST MULES AND HORSES (PACK ANIMALS AND CALVARY)
1920 TO 1945	GENEVA CONVENTION & BIOLOGICAL WEAPONS DEVELOPMENT	-1925-GENEVA CONVENTION -1935-VIRUSES FIRST OBSERVED USING ELECTRONIC MICROSCOPE. -JAPANESE USE OF BIOLOGICAL WEAPONS IN CHINA -SOVIET USE OF TULAREMIA AGAINST NAZI FORCES.
1950 TO 1969	"COLD WAR" DEVELOPMENT OF BIOLOGICAL WEAPONS BY US, UK, AND USSR	-FORT DETRICK-US -PROJECT VECTOR-USSR -PORTON DOWNS-UK
1969-1975	US UNILATERAL DISARMAMENT AND BIOLOGICAL AND TOXIN WEAPONS CONVENTION (BTWC)	1969-NIXON DISARMAMENT ANNOUNCEMENT 1972-US, UK, AND USSR START BTWC TALKS-TREATY IN EFFECT 1975.
1972- PRESENT	GENETIC ENGINEERING BEGINS	"BLACK BIOLOGY" IN USSR BIOLOGICAL WEAPONS RESEARCH.
1975-1992	USSR AND OTHER NATIONS CHEAT ON BTWC	1975-1990'S-USSR VECTOR PROGRAM; 1987-SOVIET ICBM'S LOADED WITH GERM BOMBLETS. 1978-1980'S-SOUTH AFRICA'S PROJECT COAST
1980-2001	RISE OF BIOTERRORISM	1984- Bhagwan Shree Rajneesh cult ATTACKS OF RESTAURANTS IN OREGON 1990-1995- Aum Shinrikyo-ANTHRAX AND BOTULINUM ATTACKS 2001-ANTHRAX ATTACKS IN US

SOURCES: 14, 16, 31, 32, 33, 34, 37, 39, 43, 60, 65, 66, 67

11. CITATIONS:

1- Schneider, Barry R., "U.S. Biodefense Readiness: Thoughts after September 11th." The Gathering Biological Warfare Storm. ED. Davis, J.A., Schneider, B.R. Westport, CT: Praeger, 2004.

2- Couch, Dick, Captain, USNR (retired) (2003). The U.S. Armed Forces Nuclear, Biological and Chemical Survival Manual. New York, NY: Basic Books.

3- Daly, M.J., "The Emerging Impact of Genomics on the Development of Biological Weapons: Threat and Benefits Posed by Engineered Extremophiles." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 619-630.

4 - Marty, A. M., "History of the Development and Use of Biological Weapons." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 421-434.

5 - Franz, D.R., et al. "Clinical Recognition and Management of Patients Exposed to Biological Warfare Agents." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 435-474.

6 - Burgess, T.H., et al. "Clinicopathologic Features of Viral Agents of Potential Use by Terrorists." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 475-494.

7- Martin, G. J., Marty, A. M., "Clinicopathological Aspects of Bacterial Agents." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 513-548.

8- Madsen, J.A., "Toxins as Weapons of Mass Destruction: A Comparison and Contrast with Biological Warfare and Chemical Warfare Agents." Clinics in Laboratory Medicine: Laboratory

Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 593-606.

9- Grimley, P.M., "The Laboratory Role in Diagnosis of Infections Transmitted by Arthropods." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 495-512.

10- Harris, S., "The Japanese Biological Warfare Programme: An Overview." Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945: No. 18-SIPRI Chemical & Biological Warfare Studies. ED. Zanders, J.P., Geissler, E., van Courtland Moon, J.E., New York, NY: Oxford University Press, 1999, 127-152.

11- Kagan, E., "Bioregulators as Instruments of Terror." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 607-618.

12 - Wilson, T. M., et al., "Agroterrorism, Biological Crimes, and Biowarfare Targeting Animal Agriculture: The Clinical, Pathological, Diagnostic, and Epidemiological Features of Some Important Animal Diseases." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 549-592.

13- Peterson, M.E., "Agroterrorism and Foot-and-Mouth Disease: Is the United States Prepared?." The Gathering Biological Warfare Storm. ED. Davis, J.A., Schneider, B.R. . Westport, CT: Praeger, 2004.

14- Poupard, J.A., Miller, L.A., History of Biological Warfare: Catapults to Capsomeres. *In* The Microbiologist and Biological Defense Research: Ethics, Politics and International Security. Ed. Zilinskas, R.A., Vol. 666, Annals of the New York Academy of Sciences, New York, NY, 1992. 9-20.

15- Davis, J.A., Johnson-Winegar, A., "The Anthrax Terror: DOD's Number-One Biological Threat." The Gathering Biological Warfare Storm. ED. Davis, J.A., Schneider, B.R. , Westport, CT: Praeger, 2004, 49-64.

16- Alibek, Ken, and with Stephen Handelman. Biohazard: The chilling true story of the largest covert biological weapons program in the world-told by the man who ran it. New York: Random House, 1999.

17- Shoham, D., Wolfson, Z., The Russian Biological Weapons Program: Vanished or Disappeared?. Critical Reviews in Microbiology. 30 (2004), 241-261.

18- McEleney, B.J.. "Smallpox: A Primer." The Gathering Biological Warfare Storm. ED. Davis, J.A., Schneider, B.R., Westport, CT: Praeger, 2004, 91-102.

19- Hersack, R.A., "Efficacy and Safety of the Anthrax Vaccine." The Gathering Biological Warfare Storm. ED. Davis, J.A., Schneider, B.R., Westport, CT: Praeger, 2004, 65-90.

20- Smart, J. K., "HISTORY OF CHEMICAL AND BIOLOGICAL WARFARE: AN AMERICAN PERSPECTIVE-Chapter 2." MEDICAL ASPECTS OF CHEMICAL AND BIOLOGICAL WARFARE, Eds. Sidell, F.R., Takafuji, E.D., Franz, D.R., 1997. Office of The Surgeon General at TMM Publications. 27 Jul 2008 <http://www.au.af.mil/au/awc/awcgate/medaspec/Ch-2electrv699.pdf>.

21- Wilson, C. P., "Strategic and Tactical Aerial Reconnaissance in the Near East: Military Research Papers #1." Publications. 2008. The Washington Institute for Near East Policy . 3 Aug 2008 <http://www.washingtoninstitute.org/templateC04.php?CID=54>.

22- "Levels of Command in War." Military Operations Index. 2008. Royal Australian Air Force. 3 Aug 2008 <http://www.raaf.gov.au/organisation/info_on/operations/commands.htm>.

23- "Operational Warfare." Wikipedia. 14 May 2008. Wikimedia Foundation. 3 Aug 2008 <http://en.wikipedia.org/wiki/Operational_art>.

24- Dunn , M.. "LEVELS OF WAR: JUST A SET OF LABELS? " Readings on Clausewitz, Oct 1996. Clausewitz.com . 3 Aug 2008 <http://www.clausewitz.com/CWZHOME/Dunn.htm>.

25- Caudle, L.C.. "The Biological Warfare Threat-Chapter 21." <u>MEDICAL ASPECTS OF CHEMICAL AND BIOLOGICAL WARFARE</u>, Eds. Sidell, F.R., Takafuji, E.D., Franz, D.R., . 1997. Office of The Surgeon General at TMM Publications. 27 Jul 2008 <http://www.au.af.mil/au/awc/awcgate/medaspec/Ch-21electrv699.pdf>.

26- Croddy, Eric, Perez-Armendariz, C., and Hart, J.. <u>Chemical and Biological Warfare: A comprehensive survey for the concerned citizen.</u> New York: Copernicus Books, 2002.

27- Ainscough, M.J.. "Next Generation Bioweapons: Genetic Engineering and Biological Warfare." <u>The Gathering Biological Warfare Storm</u>. ED. Davis, J.A., Schneider, B.R., Westport, CT: Praeger, 2004, 165-186.

28- Kiziah, R.R.. "Assessment of the Emerging Biocruise Threat." <u>The Gathering Biological Warfare Storm</u>. ED. Davis, J.A., Schneider, B.R., Westport, CT: Praeger, 2004, 123-164.

29- Davis, J.A.. "A Biological Warfare Wake up Call: Prevalent Myths and Likely Scenarios." <u>The Gathering Biological Warfare Storm</u>. ED. Davis, J.A., Schneider, B.R., Westport, CT: Praeger, 2004, 187-201.

30- Bullock, B. F., "Surveillance and Detection: A Public Health Response to Bioterrorism." <u>The Gathering Biological Warfare Storm</u>. ED. Davis, J.A., Schneider, B.R., Westport, CT: Praeger, 2004, 31-48.

31- Tucker, Jonathan B. (editor). <u>Toxic terror: Assessing terrorist use of chemical and biological weapons</u>. 4th printing. Cambridge, MA: MIT Press, 2001.

32- Wheelis, M., "Biological warfare before 1914." Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945: No. 18-SIPRI Chemical & Biological Warfare Studies. ED. Zanders, J.P., Geissler, E., van Courtland Moon, J.E., New York, NY: Oxford University Press, 1999, 8-34.

33- Tucker, Jonathan B.. Scourge: The once and future threat of smallpox. New York: Atlantic Monthly Press, 2001.

34- Wheelis, M., "Biological Sabotage in World War I." Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945: No. 18-SIPRI Chemical & Biological Warfare Studies. ED. Zanders, J.P., Geissler, E., van Courtland Moon, J.E., New York, NY: Oxford University Press, 1999, 35-62.

35- Koenig, Robert. The Fourth Horseman: One man's mission to wage the great war in America. New York: Public Affairs, 2006.

36- Geissler, E., "Biological Warfare Activities in Germany, 1923-1945." Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945: No. 18-SIPRI Chemical & Biological Warfare Studies. ED. Zanders, J.P., Geissler, E., van Courtland Moon, J.E., New York, NY: Oxford University Press, 1999, 91-126.

37- Mierzejewski, J. W., van Courtland Moon, J.E., "Poland and Biological Weapons." Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945: No. 18-SIPRI Chemical & Biological Warfare Studies. ED. Zanders, J.P., Geissler, E., van Courtland Moon, J.E., New York, NY: Oxford University Press, 1999, 63-69.

38- Cole, L.A., The Specter of Biological Weapons. Scientific American, 275, No.6, (1996 December), 60-65.

39- Mangold, Tom, and Goldberg, Jeff. Plague Wars: The Terrifying Reality of Biological Warfare. New York: St. Martin's Press, 1999.

40- "BIOLOGICAL WEAPONS CONVENTION: BACKGROUND INFORMATION." Disarmament: The Biological Weapons Convention. July 2008. The United Nations at Geneva. 6 Aug 2008 <http://www.unog.ch/80256EDD006B8954/(httpAssets)/699B3CA8C061D490C1257188003B9FEE/$file/BWC-Background_Inf.pdf>.

41- Burgess, S. F., Purkitt, H.E., "The Rollback of South Africa's Chemical and Biological Warfare Program." USAF Counterproliferation Center. April 2001. Air University, Maxwell Air Force Base, Alabama. 27 Jul 2008 < http://www.au.af.mil/au/awc/awcgate/cpc-pubs/southafrica.pdf>.

42- "ADDITIONAL UNDERSTANDINGS AND AGREEMENTS REACHED BY REVIEW CONFERENCES RELATING TO EACH ARTICLE OF THE BIOLOGICAL WEAPONS CONVENTION." Disarmament: The Biological Weapons Convention. July 2008. The United Nations at Geneva. 6 Aug 2008 <http://www.unog.ch/80256EDD006B8954/(httpAssets)/66E5525B50871CAEC1257188003BDDD6/$file/BWC_Text_Additional_Understandings.pdf>.

43- Miller, J., Engelberg, S., Broad, W., Germs: Biological Weapons and America's Secret War. 1st ed.. New York: Simon & Schuster, 2001.

44- Chalk, P., Infectious disease and the threat to national security. Jane's Intelligence Review. 13 (2001), 48-51.

45- Krafft, A.E., Kulesh, D.A., "Applying Molecular Biological Techniques in Detecting Biological Agents." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 631-660.

46- Henchal, E.A., et al., "Current Laboratory Methods for Biological Threat Agent Identification." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 661-678.

47- Culpepper, R.C., Pratt W.D., "Advances in Medical Biological Defense Technology." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 679-689.

48- Hersack, R.A., "Efficacy and Safety of the Anthrax Vaccine." The Gathering Biological Warfare Storm. ED. Davis, J.A., Schneider, B.R., Westport, CT: Praeger, 2004.

49- Lebeda, F.J., "Deterrence of Biological and Chemical Warfare: A Review of Policy Options." Military Medicine 162, No. 3(1997): 156-161.

50- Cerys Rees, D.G., Krieg, A. M., Titball, R.W., "Nonspecific Immunomodulator Therapy." Biological Weapons Defense: Infectious Diseases and Counterbioterrorism. Ed.. Lindler, L.E., Lebeda, F.J., Korch, G.W., Totowa, New Jersey: Humana Press, 2005, 317-331.

51- Noah, D.L., et al. "Biological Warfare Training: Infectious Disease Outbreak Differentiation Criteria." Military Medicine 163, No. 4 (1998): 198-201.

52- Wiener, S. L.. "Strategies of Biowarfare Defense." Military Medicine 152, No. 1(1987): 25-28.

53- Pravecek, T.L., Davis, J.A., "Bio-Defense Now: 56 Suggestions for Immediate Improvements-Final Report 85% Biological Defense Project." USAF Counterproliferation Center. May 2005. Air University, Maxwell Air Force Base, Alabama. 27 Jul 2008 <http://www.au.af.mil/au/awc/awcgate/cpc-pubs/pravecek.pdf>.

54- Hickman, D.C. "A Chemical and Biological Warfare Threat: USAF Water Systems at Risk." The Counterproliferation Papers, Future Warfare Series No. 3. September 1999. USAF

Counterproliferation Center. 27 Jul 2008 < http://www.au.af.mil/au/awc/awcgate/cpc-pubs/hickman.pdf>.

55- Dickey, M.E. Lt. Col. USAF (2000).Biocruise: A Contemporary Threat. The Counterproliferation Papers, Future Warfare Series. 7, 1-33.

56- Zilinskas, R.A.. "Possible Terrorist Use of Modern Biotechnology Techniques." Conference on Biosecurity and Bioterrorism. 18-19, September 2000. Landau Network . 27 Jul 2008 <http://www.mi.infn.it/~landnet/Biosec/zilinskas1.pdf>.

57- Inglesby, T.V., Grossman, R., and O'Toole, T., "A Plague on Your City: Observations from TOPOFF." Clinical Infectious Diseases, 32(2001): 436-445.

58- O'Toole, Tara. "Smallpox: An Attack Scenario." Emerging Infectious Diseases, 5 (4)(Jul-Aug 1999): 540-546.

59- Zuill, W.S.. The Story of Bermuda and her people. 3rd. London: Caribbean, 1999, pgs 141-142.

60- Mayor, Adrienne. *Greek Fire, Poison Arrows & Scorpion Bombs: Biological and Chemical Warfare in the Ancient World*. Wodstock, NY: Overlook Duckworth, 2003, pgs. 122-123.

61- Glass, Thomas A.. "Disasters and Public Health: Lessons for Bioterrorism Preparedness." Open Source Ware-Johns Hopkins Bloomberg School of Public Health course. 2007. Johns Hopkins School of Public Health. 9 Aug 2007 <http://distance.jhsph.edu/trams/index.cfm?event=training.launch&trainingID=113>.

62- Cameron, Gavin, and Jason Pate. "Covert biological weapons attacks against agricultural targets: Assessing the impact against US agriculture." Terrorism and Political Violence 13, no. 3 (Autumn 2001): 61-82.

63- McKenzie, Kenneth F. "The Revenge of the Melians: Asymmetric Threats and the Next QDR: McNair Paper 62." *Air War College, MAXWELL AIR FORCE BASE, MONTGOMERY, ALABAMA* . INSTITUTE FOR NATIONAL STRATEGIC STUDIES NATIONAL DEFENSE UNIVERSITY, 2000. Web. 28 Aug 2011.
<http://www.au.af.mil/au/awc/awcgate/ndu/mcnair62.pdf>.

64- Kolodzie, Michael L.. "Commentary: The Asymmetric Threat." Army Logistician . Jul-Aug 2001. United States Army Logisitics. 11 Aug 2007
<http://www.almc.army.mil/ALOG/issues/JulAug01/MS628.htm>.

65- Holmes, Chris. *Spores, Plagues, and History: The Story of Anthrax*. First edition. Dallas, TX: Durban House, 2003.

66- Ewald, Paul W. Plague Time: The New Germ Theory of Disease. New York, NY: Anchor Books, 2002.

67- Balmer, Brian. "The UK Biological Weapons Program." *Deadly Cultures: Biological Weapons Since 1945*. Ed. Mark Wheelis, Lajos Rozsa, and Malcolm Dando. Cambridge, MA: Harvard University Press, 2006, 47-83.

68- Preston, Richard. *The Cobra Event: A Novel*. New York: Random House, 1997. xi.

69- Black, John Logan. "Genome Projects and Gene Therapy: Gateways to Next Generation Biological Weapons." *Military Medicine*. 168.11 (2003): 864-871.

CHAPTER 4-CONCEPTS OF INTRODUCED SPECIES

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1. INTRODUCTION:

The definition of an introduced species is a non-native species introduced into a foreign ecosystem that successfully flourishes and may damage the abiotic or biotic factors of that ecosystem (1, 2). Since the introduced species usually is devoid of the 3 P's (predators, parasites, and pathogens) to the organism, the population of the non-native species increases. The terminology for an introduced species varies and can become very confusing (e.g. invasive, invader, alien, non-native, weed, etc.). Part of this confusion depends on the effects of the introduced species; either upon first entry it is ignored; considered a pest; or purposefully introduced for the benefit of mankind. Hence, for the rest of this summary a term from Colautti and MacIsaac will be used for all introduced species -Non-Indigenous Species (NIS) (3).

Examples of NIS introductions include the accidental introduction of the algae *Caulerpa taxifolia*, which has been a menace to the Mediterranean coastal ecosystem off of France, Spain, and Italy (4). The deliberate introduction into the U.S. of the black necked pheasants by Judge Owen Nickerson Denny from China; ostensively for hunting purposes (5). The accidental introduction of the Asian Long Horn beetle from wooden packing material from China; the beetle now threatens urban forests of Chicago and New York as well as threatens the lumber and maple sugar industries if it spreads (6). Some NIS organisms have hybridized with other organisms, while others have out competed the native organisms for habitat resources. NIS organisms can be viral (e.g. Foot and Mouth disease- an Aphthovirus or Yellow Fever virus carried by Asian Tiger Mosquitoes(*Aedes albopictus*), bacterial (e.g. citrus canker- *Xanthomonas axonopodis*), Protozoal (e.g. Avian Malaria-(*Plasmodium relictum*)), fungal (e.g. Chestnut Blight-*Cryphonectria parasitica*), plant (e.g. Kudzu-(*Pueraria lobata*)) or animal (examples range from the European Gypsy Moth (*Lymantria dispar*) to the Sea Lamprey (*Petromyzon marinus*)).

2. HISTORICAL EXAMPLES:

The mode of NIS introduction has varied over recorded history. In the 1800's, the Acclimatization Movements in both Europe and the United States resulted in many organisms being transported into foreign ecosystems; some perished while others flourished. Many organisms were imported into the US or Europe from various parts of the world including South Africa, Tibet, Russia, China, Africa, Indochina, Reunion Island, and Australia. Many of the well-meaning leaders and sponsors of these "acclimatization" projects never realized the effects of these NIS on human society or the ecosystems. Other NIS introductions were more accidental or unnoticed at the onset. In 1868, Leopold Trouvelot, in an attempt to breed a better silkworm imported European Gypsy Moth eggs to his residence in Medford, Massachusetts (5). The moths later escaped and the NIS became a destructive pest to forests spanning throughout the New England states and westward. The Zebra Mussel (*Dreissena polymorpha*) was originally deposited into the Great Lakes from cargo ships that emptied their ballast water that originated from Eastern Europe. The Zebra Mussel later became a major pest to Great Lake aquatic organisms as well as industries using the lake water for industrial and commercial uses. At present, the greatest invasion challenge is anthropogenic in nature. Aside of the NIS organisms that hitch a ride via cargo planes (military or commercial), inside packing materials from shipping containers, or reside in marine ballast water; other present NIS threats originate from smuggled food products, imported pets or illegal horticultural imports that evade quarantine or customs agencies (6).

3. EFFECTS OF NIS

The effects of NIS are staggering in economic, ecological, and human societal costs. In the US alone, David Pimentel estimated NIS causes $137 billion per year in losses, damage, and coastal expenses (7). This study included both economic and environmental damage, including $34 billion just for crop and forage losses and control costs for NIS. In Australia, the costs of controlling agricultural weeds alone (the majority are NIS) is $1.7 Billion per year (6). In another study, Pimentel and his team calculated the annual costs associated with NIS for the United Kingdom at $12 billion, for Brazil at $50 billion, for South Africa at $7 billion, and for India at $116 billion (8).

In the 1890's, an outbreak of Rinderpest (a Morbillivirus also referred to as "cattle plague") caused by the importation of infected Indian cattle wiped out the wildebeest and buffalo along with much of the domestic cattle in South Africa. Besides the environmental disruption caused by the loss of grazing to control bush growth in the savannas, the overgrowth provided cover for the flourishing of the tse-tse fly which led to epidemics of human sleeping sickness.

The introduction of avian malaria (via release of the mosquitoes carrying the malaria parasite) on the islands of Hawaii led to the decimation and near extinction of many rare and colorful birds on the islands (5). Another example of a NIS human societal effect (i.e. public health) is the protozoan *Cyclospora cayetanensis*. This organism sickened many US consumers in 1996 and yet was shipped in on Guatemalan raspberries (6). In this example, global market trade can transport not merely fresh produce, but NIS organisms that directly affect public health!

Several studies have further expanded the understanding of the impact of NIS on imperiled species and biodiversity. Wilcove et al (18) in 1998, analyzed the threat to US biodiversity by five categories of threats: habitat destruction, the spread of alien species (NIS), overharvesting, pollution (including siltation), and disease (including both alien and native pathogens). The analysis included data on imperiled species (including invertebrates, vertebrates, and plants) listed by the Nature Conservancy as well as endangered or threatened species in both the continental US and Hawaiian islands. The study demonstrated that NIS was the second greatest threat to imperiled species and biodiversity (second only to habitat loss). The study also supported the concept that for isolated or small land mass ecosystems (as demonstrated by the Hawaiian islands), native species from these ecosystems (e.g. plants and birds) are more imperiled from NIS invasions. The authors also noted that as the cumulative number of NIS (alien species) increases over time, NIS will have an ever-increasing threat to native plants and animals (18).

In a modeling study done in 2000 by Sala et al (19), the impacts on global biodiversity were examined by a variety of "drivers of change". These "drivers of change" are defined by the authors as the most important determinants of changes to global biodiversity. These "drivers" include:

- Changes inland use
- Atmospheric carbon dioxide concentration
- Nitrogen deposition and acid rain
- Climate
- Biotic exchanges (i.e. accidental or deliberate introduction of plants and animals to a naive ecosystem-NIS!)

The model assumes three principles which allowed the researchers to devise three possible variations of the model:

- No interactions among the various drivers of change.
- The drivers are antagonistic to the biodiversity, but that the biodiversity will respond only to the driver to which it is most sensitive.
- There are synergistic interactions and biodiversity will respond multiplicatively to the synergistic effects of the drivers.

From these three principles, three models were derived.

In the analysis across terrestrial biomes, NIS (aka biotic exchanges) had the greatest impacts on freshwater ecosystems (e.g. lakes, streams), Mediterranean and southern temperate forests. Other non-polar or non-tropical biomes (e.g. arctic and alpine ecosystems) would not be heavily impacted by NIS. Also, due to the large initial biodiversity and abiotic factors, NIS invasions would have a low probability of impact on the biodiversity of these ecosystems (19).

Finally, the study supports the concept that islands and/or small land mass ecosystems with limited biodiversity and isolation from similar habitats would be more prone to NIS invasions and disruptions of native biodiversity (19).

4. STRATEGIES TO CONTROL NIS INVASIONS:

A. ERADICATION

The strategies to control or "reverse" a NIS invasion vary depending on the biology of the organism itself; whether the NIS has established itself in the ecosystem; or whether the invasion is just beginning. Various countries and organizations have attempted the "eradication" process. Sometimes this process is successful. Eradication is most successful if the invasion of the NIS is early and yet, further depends on if the country has an early warning network and resources for a rapid response against the invasion. In essence, the rate of eradication success is proportional to the spread of the NIS (2, 4). Meinesz describes the futile efforts to rally an early response and eradication effort against Caulerpa (*Caulerpa taxifolia*) by French and Monacan bureaucrats. Eventually, the NIS (Caulerpa) had grown beyond eradication efforts (4). More successful eradication efforts include the fifty year trapping campaign by England to eradicate the South American Nutria (6). Baskin notes that "whenever eradication seems feasible, it is usually the best course to take." (6).

B. BIOCONTROL

Another strategy against NIS is biocontrol. Biocontrol is the use of another organism (predator, parasite, or pathogen) that is native for the NIS. Optimally, the biocontrol organism must be selective and feed ONLY on the NIS. If successful, this organism will reduce-yet, not eradicate-the NIS population and thus manage if not re-balance the adverse effects caused by the NIS on the ecosystem. In the past, biocontrol releases were hit or miss-sometimes exacerbating the problem. In the 1880's, mongooses were introduced onto the Hawaiian Islands to control the rats. Rats were a NIS that escaped off from trading ships over previous decades. The rats ate the native Hawaiian bird eggs and thereby threatened extinction for many of the native Hawaiian birds. Once the mongooses were released, it was discovered that the mongooses did not feed on the rats, but rather feasted on Hawaiian crows and other bird eggs. (NOTE: part of this problem is due to the fact that the rat is a nocturnal animal and the mongoose is a diurnal animal-thus, both organisms' sleep-activity cycles were opposite to each other!) (5).

Today, researchers select biocontrol agents more carefully. Usually this involves research into the native organism's (NIS) home range; selection of the biocontrol agent; study of the agent under controlled quarantine facilities; and further studies to determine if that biocontrol agent poses a threat to the target ecosystem. The key point is not merely to attack the NIS, but to prevent further damage to the biodiversity of the target ecosystem. Finally, the biocontrol organism is mass produced via controlled breeding or other means of mass producing the organism for large scale distribution into the affected ecosystem. Successful biocontrol releases include the use of the Vedalia beetle (an Australian ladybug) in 1889 to control the cottony cushion scale insect destroying the orange groves of Southern California. Within one year, the scale was barely detectable in the groves (6). More recent success in the late 1970's was the discovery and mass production of a parasitic wasp from Paraguay that attacked the cassava mealy bug. The mealy bug was a NIS in thirty African countries and destroyed 80 per cent of the cassava crop. Within less than a decade, the mealy bug was brought under control and the threat of famine was ended (NOTE: Cassava is a primary food staple in Africa) (6).

C. PREVENTION

Methods to prevent NIS introduction include various government agencies with lists that proclaim whether an organism is dirty (can become an NIS) or clean (no present threat exists). A paper by Ruesink et al (17) helped to devise the concept of a list of "dirty" versus "clean" characteristics. The challenge for many government agencies is whether to follow an "innocent until proven guilty" of being NIS strategy (aka the "dirty" list) or whether to follow a "guilty of being NIS until proven innocent" strategy (aka the "clean" list). Some countries have switched over from the dirty list which bans entry of select known NIS organisms to the clean list (which suspects all entry organisms unless they have been previous certified not to become invasive). The strategy of the "clean" list, although more frustrating to importers, horticulturists, pet hobbyists, and other collectors, nevertheless has greatly reduced the risk of another NIS entry (6).

Research done by Reichard and Hamilton (11) on the invasion of woody plant species into North America helped them to devise a predictive model in the form of a hierarchical tree that allows the user to separate species into three categories: admit (low risk of invasiveness), deny admission (high risk of invasiveness), and delay admission for further testing and intensely monitor. In this final group, if the invasiveness risk can not be fully assessed based on the included attributes, then more analysis is required before introduction should be permitted (11).

In the United States, APHIS-PPQ (Animal and Plant Health Inspection Service, Plant Protection and Quarantine) a branch of the USDA (United States Department of Agriculture), helps to control weedy introductions by controlling entry of plants and plant products. Other US agencies (such as the US Fish and Wildlife Service) work to prevent NIS incidents as well (2, 6).

Two reports developed by US government task forces (15, 16) describe the development of a risk assessment model for NIS organisms (plant or aquatic organisms) being introduced into new environments. The objective of the process (aka "the Genetic Model") was to provide a standardized process to estimate the risk of introducing NIS into new ecosystems. Initially, this model was developed by a team from APHIS, but the authors note that the risk assessment process is flexible enough to be used by other interested groups (government or otherwise) for NIS risk estimates; as demonstrated by the follow up report by the Aquatic Nuisance Species Task Force (16).

The first step (SEE FIGURE 1) is a pathway analysis which examines what important commodity may harbor the possible NIS (e.g. lumber, agricultural produce, potted plants, grain from cargo ships). Along with the data on the commodity importation, data is also collected on the high risk pathways that may result in the introduction of the NIS (e.g. waterways, ballast water, shipping crates, etc.). The analysis of the pathway and /or commodity helps to provide a Commodity/Pathway Risk Potential (CRP).

The next step is the analysis of what potential pests are associated with the imported commodity OR are located in the producing region of that commodity. The potential NIS candidates are then categorized as non-indigenous or indigenous to the United States as well as potentially capable of being a vector of another non-indigenous pest or pathogen (e.g. bacteria, fungus, parasite, rickettsia, virus, etc.). Based on the candidate list devised, an individual pest risk assessment is then devised using a two component risk assessment model.

This individual pest risk model's components are further divided into 7 basic component elements which serve to direct biological information into the assessment (SEE FIGURE 2). Each of these basic elements is represented as a probability or impact estimates which may be determined using quantitative or subjective methods. It must be noted that each element does not carry equal value in weighing of the risk nor are they necessarily independent of each other. The weight of each element is dynamic as it is strongly dependent on the NIS organism and its environment at the time of the organism's introduction (15).

The first major component is the probability of establishment and it is composed of the following four elements:

- Pest with Host/Pathway-estimate or probability of NIS organism being on or in the pathway.
- Entry potential-Probability that organism will survive the transit. This includes consideration of number of propagules in transit as well as stage of life cycle in transit.
- Colonization potential-Probability of organism colonizing and maintaining a population after introduction. This includes issues of obtaining food sources, reproductive success, and overcoming environmental resistance in the new environment.
- Spread potential-Estimate of probability of the organism spreading beyond initial colonization site. This issue includes consideration of whether the spread is by

natural means or via human activity, the development of races or strains of the organism, and the estimate range of spread of the organism over time.

The second major component of the risk assessment model for NIS is to assess consequences of establishment. This component is composed of three elements:

- Economic damage potential-Estimate the economic impact if the NIS is established. This includes damages to crops or natural resources, effects to subsidiary industries or exports and control costs.
- Environmental damage potential- Estimate of the environmental damage if the NIS is established. This includes ecosystem destabilization, biodiversity reduction or destruction, elimination of keystone species, reduction or elimination of endangered/threatened species.
- Perceived damage-Estimated impact from social and /or political influences. This includes the impact from aesthetic damage, consumer concerns, and political repercussions.

The seven risk values are combined into a final pest risk potential which represents the overall risk of the organism being assessed. This risk assessment can be combined with the Commodity/Pathway Risk Potential (CRP) which provides a final combined risk of the NIS associated with the pathway of entry.

If the risk assessment is determined to be high, the risk management team (e.g. APHIS, etc.) may determine a regulatory decision such as to prohibit entry, authorize entry, or authorize entry under specified conditions (e.g. containment lab for further monitoring and analysis, etc.) (15, 16).

It would be with noting that this risk assessment process could be used to record positive impacts that NIS might have as a biocontrol agent, new potential crop, or in scientific research (15).

In 1999, President Bill Clinton established the National Invasive Species Council as an interagency group to recommend a manageable plan of comprehensive screening plan to reduce the risk of NIS (6). Although various nations manage their own control plans and agencies, the push for globalization and free trade economics requires policies and treaties to manage the biodiversity of the world while monitoring for and preventing invasions by NIS. The

Biodiversity Treaty (section 8h) calls on member nations to prevent, monitor, and control NIS which threaten ecosystems (12). The Global Invasive Species Programme (GISP), established in 1997 after a Norway/United Nations Conference on Invasive Species, is an international consortium of organizations that works to communicate the problem of NIS as well as work on strategies to prevent or deal with NIS outbreaks (6).

5. WHAT MAKES AN NIS?

A. FACTORS

The key challenge to ecologists is to determine what factors or conditions cause an organism to become a NIS. One simple concept would be to state that a NIS is any non-native organism introduced into another ecosystem. Unfortunately, this is too simplistic, as many biotic and abiotic factors of the ecosystem can either lead to the non-native organism perishing or flourishing in the new ecosystem. For example, the abiotic conditions of temperature, salinity, or chemical composition of the water may be too extreme for the non-native organism to adapt to the new ecosystem. Furthermore, some non-native organisms may fail due to competition with native organisms as well as predators within the new ecosystem (not to mention parasites or pathogens endogenous to the ecosystem!).

Finally, some non-native organisms may succeed, but not induce disruptions within the ecosystem-either the abiotic or the biotic component. Some NIS may even develop hybrids with the native species. For example, some studies describe the hybridization of the North American Mallard with both the New Zealand gray duck and the Hawaiian duck (2, 6).

B. SLEEPER ISSUE

Another problem with the determination of NIS characteristics of an organism is the potential to be a “sleeper” NIS organism. The organisms could be introduced into the ecosystem, but may exhibit NIS behavior only after a lag time. This “lag time” could be years. Generally, the longer a species is established in an ecosystem, the higher the probability of it growing out of control and becoming a NIS (6). Baskin mentions that detection and surveillance efforts for sleeper weeds have been recently implemented in South Africa, Micronesia, Hawaii, Australia, and New Zealand (6). Unfortunately, the sleeper issue will further complicate monitoring efforts and tax limited NIS control budgets for many countries.

6. INDICATORS OF NIS POTENTIAL

A. HISTORY

Baskin discusses that one of the prime indicators of NIS potential for any organism is history. That is, the previous history of the organism in other non-native ecosystems (2, 6). If the organism has a previous "track record" of becoming a NIS in one non-native ecosystem, then it is highly probable that the same organism will become a NIS in other non-native ecosystems.

Other studies note that two major factors for successful NIS are factors within that species and factors within the non-native ecosystem. These factors for the species include whether the species can reproduce both sexually and asexually (2). Meinesz notes that the strain of Caulerpa to proliferate in the Mediterranean Sea was growing quickly as small fragments of the algae were breaking off, drifting in the currents, and starting new organisms distant from the origin of infestation (4). Normally, Caulerpa in its native environment (tropical Atlantic Ocean) exhibits sexual reproduction. Other factors for NIS behavior include broad tolerance to environmental parameters (e.g. temperature, salinity); or as with some plants-the trait of allelopathy (such as producing growth inhibitors in the root to inhibit the growth of native plants); initial resistance to native parasites; aggressive nature; or the capacity to produce large seed clusters in short intervals (2, 4).

B. ECOSYSTEM FACTORS

The ecosystem factors of the non-native ecosystem that would favor a successful NIS invasion include disruption of the ecosystem; reduced biodiversity within the ecosystem; and a vulnerable ecosystem that has remained geographically or evolutionarily isolated (e.g. islands) (2, 6). Common disruptions of ecosystems include roads, natural disasters (like fire, droughts, or floods), and polluted ecosystems. It is interesting to note that Meinesz stated that the Caulerpa did in fact grow well in polluted waters (4)! Studies indicated that species diversity appears to promote stability within the ecosystems and thus can ward off NIS invasions (6). This biodiversity within ecosystems could be referred to as "ecological resistance" to the invader. Therefore, using this reasoning, those sites with the MOST limited biodiversity-such as agriculture fields with monoculture would be very suspect to NIS invasions.

C. DISRUPTED HABITATS

Several studies support this reasoning that habitats that are more disturbed are more vulnerable to NIS invasions. Hansen and Clevenger (13) observed that in transportation corridors which create disturbance regimes in plant communities along the corridor edges, the probability of invasive species establishing and spreading is greatly increased as compared to control sites or to habitats a significant distance from the corridor site. Mack and D'Antonio (14) reviewed various studies of human activities and the intensity of ecological disturbances. One interesting additional observation the authors reported was that human activities could disturb ecosystems by the introduction of invasive species (14). The studies indicate that NIS modification can restructure the ecosystems by modifying disturbance regimes or adding new disturbances to the ecosystems. It is worthy of noting that the authors concluded that even the modifications of disturbance regimes resulted in a transition of ecosystems into a new state of structure and function (14).

Finally, the most vulnerable ecosystems are those isolated ecosystems. In many texts, the Hawaiian and Galapagos islands are prime examples of ecosystems with a large biodiversity (yet isolated!) and yet have been threatened by NIS invasions (5, 6) ranging from feral cats, pigs, rats, and dogs, to avian malaria, mongooses, and even goats.

D. PROPAGULE PRESSURE

Another concept that has contributed to further understanding of NIS development has been the concept of "Propagule Pressure" (6). Propagule Pressure focuses on the number of invading "propagules" (i.e. organisms, whether it is fragments of Caulerpa or bacteria cells, or mosquitoes, or Zebra mussel larvae, or feral pigs) for a given introduction AND the frequency with which they are introduced; that is, how many times were the organisms introduced to the ecosystem! (10, 11). Various studies have indicated that Propagule Pressure is a strong predictor of NIS success (6). It must be kept in mind that it is not merely the number of organisms, BUT the number of times the delivery of organisms occurs. Also, Verling and associates note that each delivery may not always means the same number of organisms delivered and thus the variability may play a role in the eventual success of the invasion (11).

Finally, with Propagule Pressure, Colautti and MacIsaac (5) were able to not merely develop a "neutral terminology" for NIS invasions, but to develop an objective framework for invasions that describes the invasion from a biogeographic approach rather than from other approaches (e.g. taxonomic, etc.). Also, the authors suggest a framework model for invasion studies that describes the stages of invasion as well as takes into account the various filters (e.g. environmental suitability, community suitability, reproduction, dispersal, etc.) and the NIS abundance and distribution during the invasion process. Thus, the model helps to guide future studies using the Propagule Pressure concept and perhaps illuminate the factors that influence NIS development (2, 6).

E. HYPOTHESES FOR SUCCESSFUL NIS INVASIONS:

Several hypotheses have been developed to try to explain successful NIS invasions. These hypotheses have been followed with experiments. Since the multi-stage process of transport, establishment, and demographic expansion all factor into NIS success; some studies note the difficulty in testing the establishment phase and hence many focus on the post-establishment demographic expansion as this can provide an indicator of the abundance and impact of the NIS (20). Other studies use contained environments (e.g. pots of native versus naïve soils for NIS plants) to determine NIS success. Furthermore, it must be stated that not all hypotheses are mutually exclusive to each other, and some hypotheses maybe more supported for some selective NIS invasions.

One hypothesis is similar to the earlier discussed concept (absence of the 3P's-parasites, pathogens, and predators). In a review by Torchin and Mitchell (20), the authors refer to all pathogens and parasites as "parasites for plant NIS" in their studies. The author's paper demonstrated that a number of NIS were able to flourish as they have little or no parasites in the new ecosystem. The authors further note that even for new fungal and viral pathogens for the NIS (plant or animals) accumulated in the new environment, it was only a fraction (13% to 25%) as many as the NIS had in its native ecosystem (hence, NIS escape from parasites). Furthermore, NIS plants and animals should be more completely released from specialist natural enemies than from generalist enemies. Therefore, the NIS will still have a competitive dominance against native competitive species. This absence of biotic resistance can help to explain the increased demographic expansion (e.g. increased population size or biomass). If so, then the shift in influencing demographic success of NIS relies less on biotic factors and more on abiotic or biogeographic factors (e.g. temperature, rainfall, soil pH, etc.). These factors may play a key role in the predictiveness of NIS expansion via modeling-which will be discussed further in a subsequent chapter (20).

One further study by Mitchell and Power (21) explored two hypotheses as to why NIS have had severe impacts on ecosystems. The Enemy Release Hypothesis argues that NIS impacts results from reduced enemy attacks, whereas the Biotic Resistance Hypothesis argues that interactions with native species (the three P's) limit the impact of NIS. Mitchell and Power tested the hypotheses for virus and fungal pathogens that infect 473 NIS plant species naturalized to the US from Europe. Their study found that 84% fewer fungi and 24% fewer virus species infected the NIS plants in the new ecosystem as compared to their native ecosystems. Furthermore, the NIS plants that were more completely released from pathogens were more likely to be reported as harmful invaders of both agricultural and natural ecosystems. This study supports the Enemy Release Hypothesis, but the authors note that evidence of NIS plants that accumulated more pathogens in the non-native ecosystems were less likely to be noxious species. Hence, BOTH hypotheses were supported (21).

Furthermore, Blumenthal et al (36) reported in a study which reviewed 243 European plant species (NIS) released into the United States that the the release from enemies and resource-rich environments may synergistically enhance invasion success in some NIS plant species. The authors examined the success of the NIS from pathogen release (enemy release) as well as resource availability in the naïve niche. Their findings reported that fast growing NIS species benefitted most from enemy release in resource rich environments and that both factors were synergistic to the invasive success of the NIS (36). Yet, Funk and Vitousek (37) found that in low nutrient niches, plant invaders (NIS) succeeded by employing resource conservation traits, commonly referred to as high resource use efficiency strategies, such as more efficient capture of light, direct capture of nitrogen (from the air), or more efficient carbon assimilation per unit of resource. Seastedt (38) observes that even with the benefit of less enemies in the naïve habitat, the higher efficiencies of NIS in the nutrient poor niches enhance the NIS success in these habitats.

It is important to note that a study by Torchin et al (22) performed a similar study on NIS animals (using 26 NIS species including Molluscs, Crustaceans, Fishes, Birds, Mammals, Amphibians, and Reptiles) and examined their parasite release on non-native ecosystems. In summary, the NIS populations had roughly half of the parasite species in the non-native ecosystems as compared to the native ecosystems. The authors note that parasite success is hindered in NIS species as introduced populations often are derived from small subset populations and some are derived from uninfected life history stages. Another variable to limited parasite success is that many parasites have complex life cycles requiring more than one host. If the new ecosystem lacks suitable hosts for the parasite life cycle or a particular parasite life cycle, then the success of the parasite is very low. As the absence of NIS parasites exist in the non-native ecosystem, this may contribute to the enhanced success of the NIS (22).

Blossey and Notzold (23) in 1995 introduced the Evolution of Increased Competitive Ability (EICA) hypothesis to help explain NIS success in non-native ecosystems. In essence, NIS invaders in alien (non-native) environments tend to have more biomass (e.g. vegetative growth) and produce more seeds as they are in favorable environments and freed from resource reliance for herbivore defense. Since herbivore defense uses up energy and plant resources, NIS traits that are no longer needed are not favored and these resources and energy can be dedicated for enhanced competitive success in the new ecosystem. As a result, competitive abilities (i.e. NIS invasiveness) can be maximized by increased vegetative growth or reproductive efforts depending on which is more important for success in the non-native environment. Using Purple Loosestrife (*Lythrum salicaria L.*), the researchers notes that plant biomass and height were greater in potted plants grown in non-native Ithaca, NY USA as compared to the native ecosystem in Lucelle, Switzerland (23).

In 2004, Withgott (24) noted that a series of 14 papers tested the EICA hypothesis. The author notes that five studies supported the hypothesis, one rejected it, and the remainder were inconclusive. It is possible that the circumstances for various NIS species (plants, animals, etc.) may vary in analyzing this hypothesis.

One study which contradicted the EICA hypothesis attempted to extend beyond the hypothesis. Bossdorf et al (25) examined garlic mustard (*Alliaria Petiolata*), an European herb that has become an NIS in North American deciduous forests. Using EICA hypothesis reasoning, invasive genotypes should be more competitive and therefore out complete their ancestors from the native ecosystem range. The Bossdorf et al (25) study compared Garlic Mustard samples from the non-native population to the native population in competitive plantings in greenhouses. The study found that native (European) Garlic Mustard plants paired against invasive (American) plants were more competitive in biomass and other plant fitness variables. The authors concluded that the data supports a new hypothesis, Evolutionary Reduced Competitive Ability (ERCA). This hypothesis states that if there is less competition in the invasive range and the competitive ability involves traits that have a fitness cost (such as competition within a number plants of the same species), then selection might act against it. With the reduction in resource competition within a species, the savings would be used to contribute to invasion success by other means such as plasticity, tolerance to herbivory, or allelopathy (25).

This last term leads us to the next major hypothesis: novel weapons. Research papers by Callaway et al (26, 27) defines the novel weapons hypothesis as NIS that obtain invasive capability by exuding biochemicals that are highly inhibitory (i.e. allelopathic) to plants or soil microbes in invaded communities. It must be noted that these same weapons are relatively ineffective against native neighbors that have evolved over time along with the NIS in there native ecosystems. Callaway et al notes that this process many explain how NIS organisms are weak in their native ecosystem, but can be aggressive invaders in non-native ecosystems (26). Callaway et al notes that Garlic Mustard suppresses fungal mutualists in North American soils (27). Inderjit et al (28) notes that NIS studies that rely on the biogeographic approach often miss the mechanistic effects of allelopathy of NIS.

Bais et al (29) observes that the phytotoxin (-)-catechin released by the Spotted Knapweed (*Centaurea maculosa*) triggers a wave of Reactive Oxygen Species (ROS) at the native plant root meristem leading to the death of the root system. Further studies by He et al (30) found that Spotted Knapweed phytotoxic effects were very effective on non-native species more than native European plant species. Inderjit et al (31) notes that the phytotoxic effects of (+-)-catechin vary in vitro, in soil, and in field studies; yet, in all studies (+-)-catechin was very inhibitory to test plants at very low concentrations (e.g. 40 micrograms per liter soil). Vivanco et al (32) describe how the NIS Diffuse Knapweed (*Centaurea diffusa*) produces a phytotoxic root exudate, 8-hydroxyquinoline. The effects of this phytotoxin vary biogeographically in soil concentration and in its effects as an allelochemical. Diffuse Knapweed has a stronger effect on native grasses from North America than on native range grasses from Europe (32).

Callaway et al (26) notes two important points regarding the novel weapons research (33). The allelopathic effects of the European invaders (e.g. Spotted Knapweed and Diffuse Knapweed) may have differing and more subtle effects on European soil microbes (including pathogens) and native plants due to the coevolutionary history of these organisms. Hence, when these NIS knapweeds are introduced into non-native ecosystems, the flora and soil microbes with no previous history of exposure are inhibited and the NIS can succeed. Callaway also notes that some surviving soil-borne pathogens in the non-native ecosystem; for example, the generalist fungi *Sclerotinia sclerotiorum*, can proliferate in the soil community disrupted by the phytotoxin catechin and therefore become a pathogen to Spotted Knapweed. It is also worth noting that as soil ecosystems become disrupted and plant pathogens increase in biomass, the spread of plant pathogens could also affect the native plants while providing a competitive advantage to the spread of the NIS.

Callaway's work in novel weapons may help explain the time lag phase (aka sleeper period) observed in some NIS species prior to colonization or expansion. With NIS releasing allelopathic chemicals, the concentration of the root exudates and rate of the build up in the soil may account for a lag phase before the biological inertia by the resident community species (as well as the soil microbe ecosystem) is adversely affected. Callaway notes that aside of allelopathic effects of phytotoxins, other factors such as the dynamics of exponential population growth (usually seen in successful NIS invasions) from the initial growth may demonstrate a lag phase (33).

Callaway et al (34) further discusses that limits to NIS invasion may involve evolved tolerance to the invaders novel allelochemistry. Studies compared experienced versus naïve populations exposed to Knapweed invasions, and hence catechin, demonstrated some selection for allelochemical tolerance. Therefore, the evidence of the selection of tolerant strains for native species indicates that limits to invasion by NIS species exhibiting novel weapons is possible (34).

Finally, Bulleri et al (35) discusses the concepts that positive interactions between species must be considered in predicting NIS invasions of ecosystems. The authors describe two possible relationships of positive interactions with NIS invasions. Facilitation is the biotic interaction where at least one species involved benefits from the presence of others and neither is negatively affected. The presence of one species can facilitate directly by altering environmental conditions or indirectly by lessening competitive or consumer pressure. Bulleri et al (35) note that even when resources are monopolized by a small number of species or groups, invisibility can be sustained by facilitation.

It is possible that extant exotic species can enhance colonization of new exotics (e.g. invasion meltdown). Yet, this concept supports the previously described concept of “propagule pressure”. If a genetically diverse NIS population repeatedly enters a non-native ecosystem, then the initial colonizers may enhance survival of subsequent propagules within the NIS species. But, if an NIS has altered the ecosystem, subsequent NIS species invasions (same species or a different species) maybe enhanced by the altered ecosystem’s biotic or abiotic components.

Simberloff and Von Holle (39) reviewed 254 scientific articles on NIS invasions to determine evidence of an NIS organism having had a positive impact on the invasion success of another NIS organism. The authors found 10 recorded instances of positive effects (direct or indirect) on NIS influencing other NIS invasions. These influences included animal pollination and/or dispersing of NIS plants as well as NIS animals or plants modifying habitats which then favored the invasions of other NIS organisms into the habitat (39). The authors noted little evidence of NIS impeding the success of subsequent NIS invasions. The authors defined the term “Invasional Meltdown” as the synergistic interactions among NIS invaders which leads to accelerated impacts on the naïve ecosystem (39).

In the Fluctuating Resource Hypothesis, Bulleri et al (35) predict that significant fluctuations in resource availability will enhance community invasibility (NOTE: the authors define invasibility as the ability of NIS to colonize and thrive in new habitats). An NIS must have access to resources (e.g. oxygen, nutrients, light, water, etc.) and that the NIS will obtain greater success in community invasion if it does not encounter intense competition for these resources from native species. Resource availability increases if the resource supply rate to the ecosystem is increased beyond routine biotic demands or if the native biotic demand for the resources declines (as in ecosystem disruption regimes). The authors conclude that invasion scenarios and management strategies based on mere negative species interactions will limit predictive models and problem solving strategies.

The authors urge that efforts to construct a unified theory of invasibility must include attributes of native AND invading species that would provide an analysis of BOTH the positive and negative interactions (35).

Further research into NIS hypotheses will require gene expression studies to determine how NIS organisms differ from the native to the non-native ecosystems as well as how the genome of native organisms (i.e. competitors) are affected by NIS invasions. It would be interesting to investigate whether NIS genetic expression of defense traits are reduced or increased by introduction into non-native ecosystems and what are the mechanisms to alter gene expression.

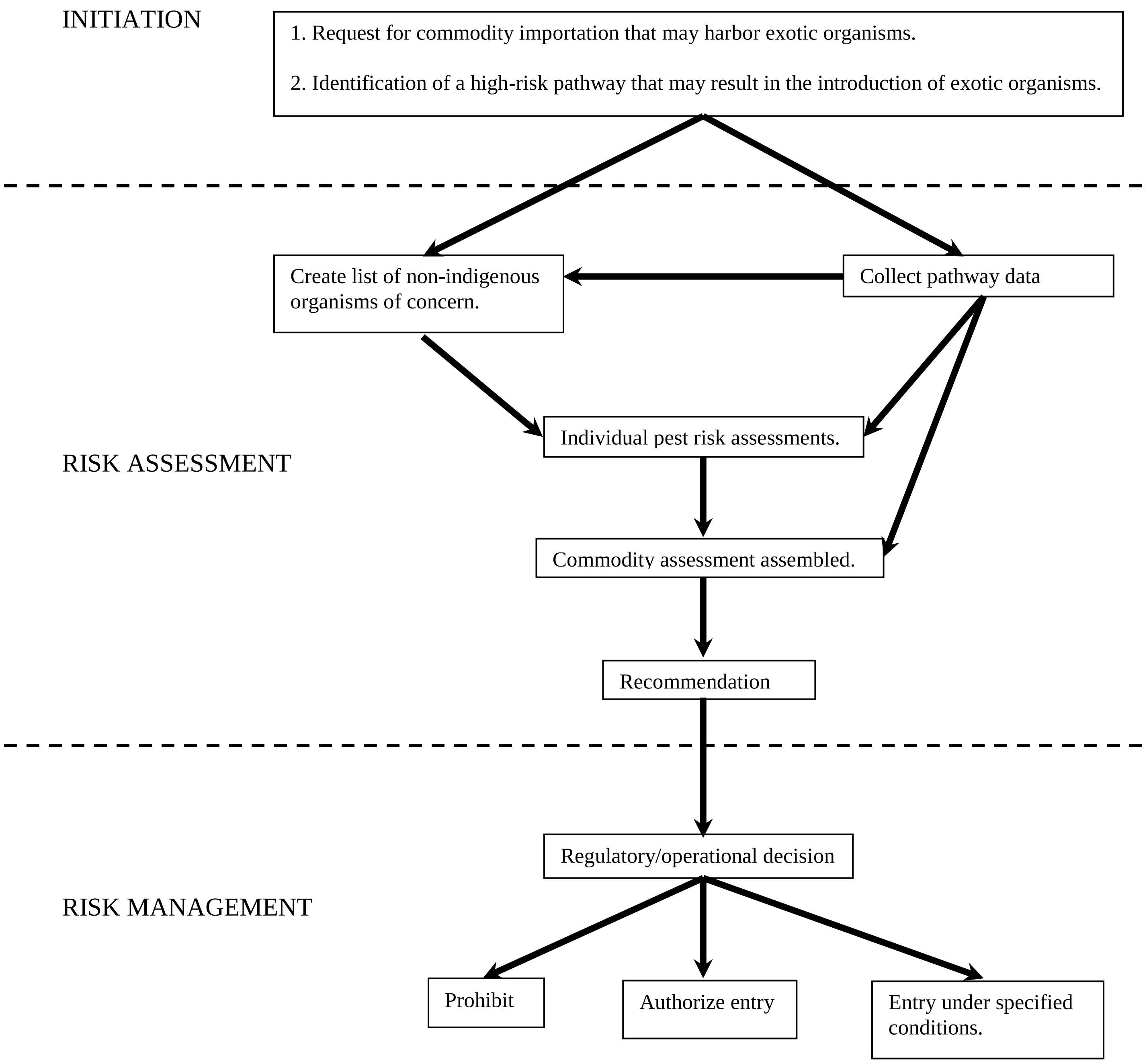
7. SUMMARY

Non-Indigenous Species (NIS) (aka introduced species) are organisms that enter foreign ecosystems, colonize, and spread while damaging the biotic or abiotic factors of the non-native ecosystem. The means of introduction can vary from accidental to deliberate and in many cases, a human factor is part of the introduction. The effects of NIS invasions are staggering in economic, ecological, and human societal costs. Studies further indicate that NIS invasions have had and will continue to have a significant negative impact on the biodiversity of global biomes. The methods to stop or prevent NIS invasions include eradication, biocontrol, and development of risk assessment protocols on organisms prior to their introduction into new ecosystems. The factors that contribute a role to NIS invasion, colonization, and expansion include the following examples: dual modes of reproduction (asexual and sexual), allelopathy, broad tolerance to environmental parameters, absence to parasites, pathogens or predators (aka 3P's) in the non-native ecosystem, and high reproduction capacity (e.g. seeds, short gestation periods for offspring, etc.). Environmental factors that may favor successful NIS invasions include, disruption regimes of the ecosystem, reduced biodiversity, small islands or isolated small land mass ecosystems. Further biogeographic modeling concepts using computer algorithms will be discussed in a later chapter

Models and hypotheses to determine NIS potential include prior history of NIS invasiveness, propagule pressure (number of invading propagules), Enemy Release hypothesis (absence of the 3P's), Biotic Resistance hypothesis (NIS limited by 3P's of native species), EICA hypothesis (NIS in non-native ecosystems favors genetic resources for maximum competition and expansion over resources dedicated to herbivore defense), ERCA hypothesis (NIS competition within the species is costly to fitness, but in non-native ecosystems, the costs are reduced and the NIS resources can be directed to invasion success and colonization), and novel weapons (allelopathic compounds produced by the NIS are used to disrupt competition from native plants and soil microbes). Finally, two hypotheses focus not on negative interactions caused by NIS, but on positive interactions of the NIS species with species of the non-native ecosystems. Facilitation is the biotic interaction where at least one species involved benefits from the presence of the other and neither is negatively affected. The Fluctuating Resource hypothesis predicts that significant fluctuations in resource availability will enhance community invasiveness.

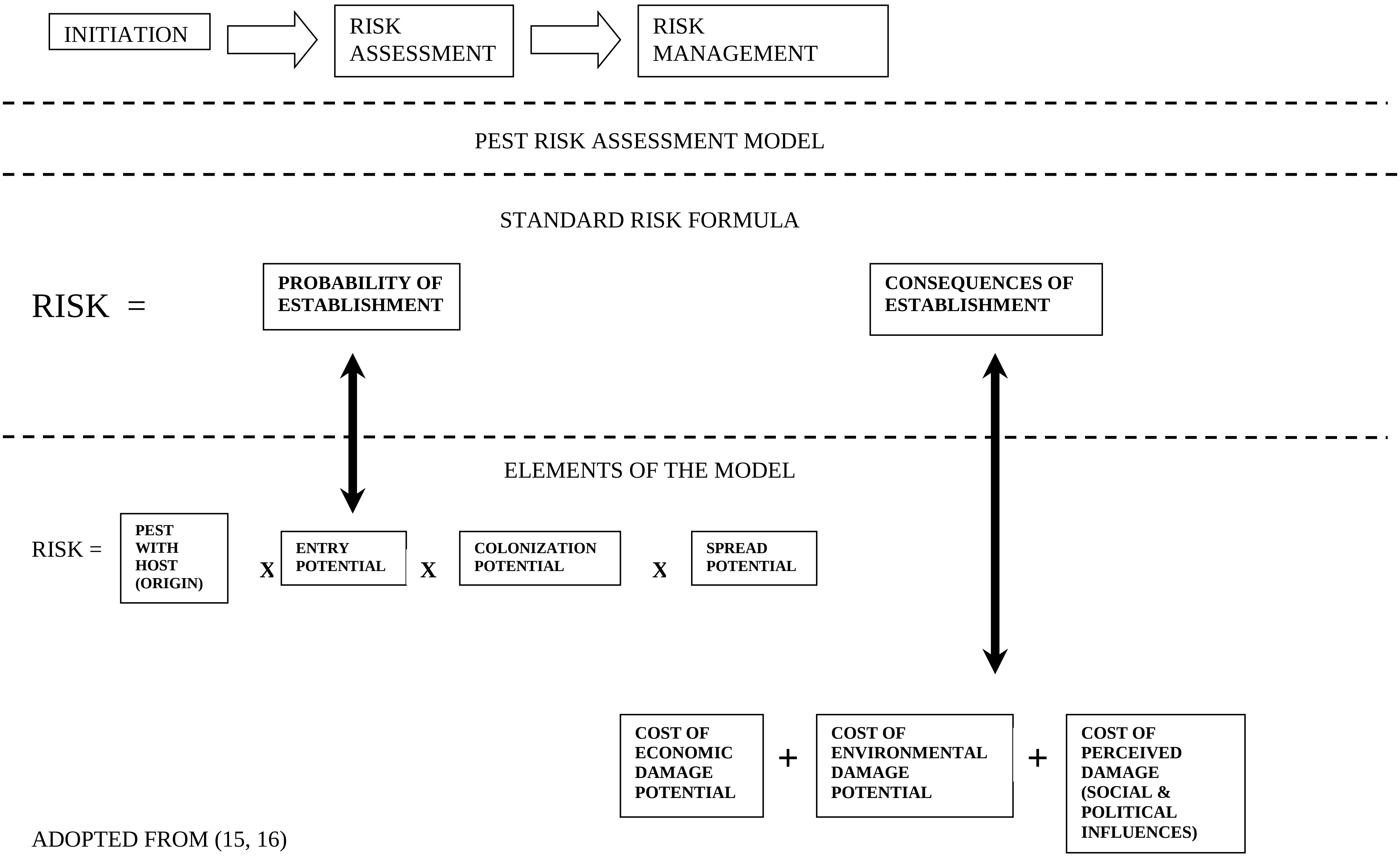
As the global markets import and export goods and services across the globe (e.g. fruit, packing crates, grain, lumber, Christmas trees, wicker furniture, etc.), US and International organizations (e.g. APHIS, USDA, EPA, United Nations, World Trade Organization) continue their attempts to curb NIS invasions. New research efforts will continue to discover new NIS invasions as well as study the means to prevent or eradicate NIS invasions. These subsequent studies will further illuminate prevention strategies, biocontrol methods, and containment efforts as well as help expand or develop NIS development hypotheses and predictive models for ecosystem NIS susceptibility. It is these tools that will contribute to stifling NIS invasions globally as well as hopefully lead to further understanding (and future warnings) of NIS development in various ecosystems across the globe.

FIGURE 1: PATHWAY ANALYSIS" FLOWCHART DEMONSTRATING INITIATION, RISK ANALYSIS, AND RISK MANAGEMENT FOR A PATHWAY



ADOPTED FROM (15, 16)

FIGURE 2: PEST RISK ASSESSMENT MODEL



ADOPTED FROM (15, 16)

9. CITATIONS:

1- "What is an Invasive Species? "*National Invasive Species Information Center*. 24 Aug 2009 . National Agricultural Library, USDA, Web. 14 Dec 2009. <http://www.invasivespeciesinfo.gov/whatis.shtml>.

2-*Predicting Invasions of Nonindigenous Plants and Plants Pests*. National Research Council. Washington, DC: National Academy Press, 2002.

3- Colautti, Robert I., and Hugh J. MacIsaac. "A neutral terminology to define 'invasive' species." Diversity and Distribution 10 (2004): 135-141.

4- Meinesz, Alexandre, and Translated by Daniel Simberloff. Killer Algae. Chicago: University of Chicago Press, 1999.

5- Todd, Kim. Tinkering with Eden: A natural history of exotic species in America. New York: W. W. Norton & Company, 2001.

6- Baskin, Yvonne. A plague of rats and rubber vines: The growing threat of species invasions. Washington, DC: Island Press, 2002.

7- Pimentel, David, et al. "Environmental and economic costs of nonindigenous species in the United States." BioScience 50, No. 1(2000): 53-62.

8- Pimentel, David, et al. "Economic and environmental threats of alien plant, animal, and microbe invasions." Agriculture, Ecosystems, and Environment 84 (2001): 1-20.

9- Williamson, M., and Fitter, A. "The characters of successful invaders." Biological Conservation 78 (1996): 163-170.

10- Verling, Emma, and et al. "Supply-side invasion ecology: characterizing propagule pressure in coastal ecosystems." Proceedings of the Royal Society B 272 (2005): 1249-1257.

11-Reichard, Sarah Hayden, and Hamilton, Clement W. "Predicting Invasions of Woody Plants Introduced into North America." *Conservation Biology*. 11.1 (2002): 193-203.

12-"Article 8. In-situ Conservation ." *The Convention on Biological Diversity*. 30-Mar-2007. United Nations, Web. 14 Dec 2009. <http://www.cbd.int/convention/articles.shtml?a=cbd-08>.

13-Hansen, Malin J., and Clevenger, Anthony P. "The influence of disturbance and habitat on the presence of non-native plant species along transport corridors." *Biological Conservation*. 125.2 (2005): 249-259.

14-Mack, Michelle C., and D'Antonio, Carla M., "Impacts of biological invasions on disturbance regimes." *Trends in Ecology & Evolution*. 13.5 (1998): 195-198.

15-Orr, R.L., Cohen, S.D., and Griffin, R.L., *Generic Non-Indigenous Pest Risk Assessment Process*. Beltsville, MD: U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 1993.

16-Orr, Richard, and et al. "Report to the Aquatic Nuisance Species Task Force." *Generic Nonindigenous Aquatic Organisms Risk Analysis Review Process*. Bethesda, MD: Aquatic Nuisance Species Task Force, 1996.

17- Ruesink, J.L., I.M. Parker, M.J. Groom, and P.M. Karelva. "Reducing the Risks of Nonindigenous Species Introduction." *BioScience*. 45.7 (1995): 465-477.

18-Wilcove, David S., Rothstein, David, and et al. "Quantifying Threat to Imperiled Species in the United States." *BioScience*. 48.8 (1998): 607-615.

19-Sala, O.E., F.S. Chapin, J.J. Armesto, and et al. "Global Biodiversity Scenarios for the Year 2100." *Science*. 287.5459 (2000): 1770-1774.

20-Torchin, M.E., and C.E. Mitchell. "Parasites, Pathogens, and Invasions by Plants and Animals." *Frontiers in Ecology and the Environment*. 2.4 (2004): 183-190.

21-Mitchell, C.E., and A.G. Power. "Release of Invasive Plants from Fungal and Viral Pathogens." *Nature*. 421. (2003): 625-627.

22-Torchin, M.E., K.D. Lafferty, and et al . "Introduced Species and their Missing Parasites." *Nature*. 421. (2003): 628-629.

23-Blossey, Bernd, and Notzold, Rolf. "Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis." *Journal of Ecology*. 83. (1995): 887-889.

24-Withgott, Jay. "Are Invasive Species Born Bad?." *Science*. 305.5687 (2004): 1100-1101.

25-Bossdorf, Oliver, and Prati, Daniel, et al. "Reduced competitive ability in an invasive plant." *Ecology Letters*. 7. (2004): 346-353.

26-Callaway, Ragan, and W.M. Ridenour. "Novel weapons: Invasive Success and the Evolution of Increased Competitive Ability." *Frontiers in Ecology and the Environment*. 2.8 (2004): 436-443.

27-Callaway, R.M., D. Cipollini, and et al. "Novel Weapons: Invasive Plant Suppresses Fungal Mutualists in America but not in its native Europe." *Ecology*. 89. 4 (2008): 1043-1055.

28- Inderjit, T.R. Seastedt, and et al. "Allelopathy and Plant Invasions: Traditional, Congeneric, and Bio-Geographic Approaches." *Biological Invasions*. 10. (2008): 875-890.

29-Bais, H.P., R. Vepachedu, and et al. "Allelopathy and Exotic Plant Invasion: From Molecules and Genes to Species Interactions." *Science*. 301. (2003): 1377-1380.

30-He, Wei-Ming, Yulong Feng, and et al. "Novel Weapons and Invasion: Biogeographic differences in the competitive effects of *Centaurea maculosa* and its root exudate (+-)-catechin." *Oecologia*. 159. (2009): 803-815.

31-Inderjit, J.L. Pollock, and et al. "Phytotoxic Effects of (+-)-Catechin in vitro, in Soil, and in the Field." *PLOS ONE*. 3.7 (2008): e2536, doi:10.1371/journal.pone.0002536.

32-Vivanco, J.M., H.P. Bais, and et al. "Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion." *Ecology Letters*. 7. (2004): 285-292.

33-Callaway, R.M., J.L. Hierro, and A.S. Thorpe, "Evolutionary trajectories in plant and soil microbial communities: *Centaurea* invasions and the geographic mosaic of coevolution." IN: *Exotic Species Invasions: Insights into Ecology, Evolution and Biogeography.* Eds. Sax, D.F., Gaines, S.D., and Stachowicz, J.J., Sinauer, Sunderland, Massachusetts USA, 2005, 341-363.

34-Callaway, R.M., W.M. Ridenour, and et al. "Natural Selection for Resistance to the Allelopathic Effects of Invasive Plants." *Journal of Ecology*. 93. (2005): 576-583.

35-Bulleri, F., J.F. Bruno, and L. Benedetti-Cecchi. "Beyond Competition: Incorporating Postive Interactions between Species to Predict Ecosystem Invasibility." *PLOS Biology*. 6.6 (2008): e162. doi:10.1371/journal.pbio.0060162.

36-Blumenthal, Dana, Charles E. Mitchell, and ET AL. "Synergy between Pathogen Release and Resource Availability in Plant Invasion." *Proceedings of the National Academy of Science*. 106.19 (2009): 7899-7904.

37-Funk, Jennifer L., and Peter M. Vitousek. "Resource-use Efficiency and Plant Invasion in Low-resource Systems." *Nature*. 446. (2007): 1079-1081.

38-Seastedt, Tim. "Resourceful Invaders." *Nature*. 446. (2007): 9885-986.

39-Simberloff, Daniel, and Betsy Von Holle. "Positive Interactions of Nonindigenous Species: Invasional Meltdown?." *Biological Invasions*. 1. (1999): 21-32.

CHAPTER 5. COMPARISON OF THE ROLE OF INFECTION BY PATHOGEN VERSUS INTRODUCTION OF INVASIVE SPECIES

CHAPTER 5: COMPARISON OF THE ROLE OF INFECTION BY PATHOGEN VERSUS INTRODUCTION OF INVASIVE SPECIES

1. INTRODUCTION

This section explores the similarities of the introduction of an invasive species (AKA Non-Indigenous Species-(NIS)) (1) into an ecosystem with the introduction of a pathogen into an organism (e.g. human, animal, or plant). Note: the term NIS which was derived from Colautti and MacIsaac (1) will be used for all invasive species. Mack et al (16) refers to this analogy concept between epidemics caused by parasites and NIS invasions as many critical factors in disease epidemiology have direct parallels in the study of NIS invasions.

Several similarities exist and these comparisons are certainly not exclusive nor complete. It is worth noting that two key concepts: time lag and multiple propagules are concepts that have parallels with both disease pathogenesis and NIS invasions (and NIS colonization). Furthermore, it is interesting to note how pathogens can evade immune processes of an organism and how the NIS can release compounds to enhance its colonization in an ecosystem. Another topic explored is that early detection of pathogen infections and NIS introductions can lead to counterstrategies to eradicate the infection (pathogen) or infestation (NIS). This chapter also explores one other important issue that is key to success of pathogens or NIS as biowarfare agents: population dynamics of NIS invasion.

Yet, it must be noted other abiotic and biotic factors play a role in the successful "infection" by either pathogens or NIS. These will be discussed in this and succeeding chapters. Admittedly, complete understanding of all of the abiotic and biotic factors that help determine the success of a pathogen or NIS against a host organism or ecosystem respectively, will require more research on an organism by organism basis.

Finally, the comparison of pathogen and NIS use as a bioweapon requires a brief discussion on the risk factors of organisms "backfiring". That is, the agent released by a hostile force onto a target may in fact return to injure or kill the original hostile force's personnel or ecosystem.

2. MULTIPLE PROPAGULES VERSUS MINIMUM NUMBER OF PATHOGEN CELLS (OR MINIMUM DOSAGE OF TOXINS)

The concept of Multiple Propagules (AKA Propagule Pressure) is defined as (1, 2, 3) the number of invading "propagules" (i.e. organisms, whether it is fragments of Caulerpa or bacteria cells, or mosquitoes, or Zebra mussel larvae, or feral pigs) for a given introduction AND the frequency with which they are introduced; that is, how many times were the organisms introduced to the ecosystem! (4, 5). Various studies have indicated that Propagule Pressure is a strong predictor of NIS success (6, 7). It must be kept in mind that it is not merely the number of organisms, BUT the number of times that the delivery of NIS organisms occur. Also, Verling and associates note that each delivery may not always mean the same number of organisms delivered and thus the variability may play a role in the eventual success of the invasion (3, 4). Finally, with the number of propagules comes the greater number of genetic variations within the population of NIS. The greater the amount of genetic variations within the population will enhance the success of the NIS invasion and the eventual spread into the naïve niche (6).

Although this term is used in describing a process by which NIS may be introduced into a naïve ecosystem, a parallel epidemiological concept exists called the Minimum Dosage of Cells. This is the minimum number of cells required to successfully infect a host organism. Another epidemiologically related term is Infectious Dose, which is the number of microorganisms or viruses sufficient to establish an infection (17). Yet in biowarfare, one key factor (8, 9) in a pathogen organisms' selection as a bioweapon is the minimum number of cells that are required to cause illness or death to the target (e.g. human, animal, plant, etc.). The lower the number of cells required means that the nation or terrorist using the bioweapon can make less cells for an attack weapon OR that during the bioweapon distribution (e.g. as an aerosol) the weapon can have a greater impact on targets (e.g. greater distribution means greater causalities) at lower dosages during the aerosol distribution (8, 9). Franz et al (9) notes that some bioweapons agents have an extremely low infectious dose (minimum number of cells), such as *Coxiella burnetii* (agent of Q Fever) is 1 to 10 inhaled organisms or Yersinia pestis (agent of Plague) requires only 100 to 500 inhaled organisms to achieve infection. Variations for the minimum infectious dosage of cells (or viruses or toxin molecules for other biowarfare agents) depends on the roles of the pathogens' virulence factors as well as the resilience of the organism to survive distribution as an aerosol (i.e. aerobiological factors) or as a waterborne agent as well as pathogenic strategies to overcome or evade innate and/or adaptive immunity of the host (8, 9, 10, 11). For example, Yershov et al (16) explored the infectious dose of *Streptococcus pneumonia* to cause pneumonia in a rabbit models. The study found that as increasing dosages of *S. pneumonia* cells are inoculated into the rabbit endobronchially, a threshold of 4.67 log 10 CFU was found. This threshold is the infectious dose which exceeds the antibacterial protection by the rabbit respiratory system and made the development of pneumonia in rabbits more predictable (up to 90%) (16). This process parallels the multiple propagules concept as a certain threshold (here the number of *S. pneumonia* bacteria cells) was determined to be necessary to overwhelm the antibacterial protection of the lungs and achieve infection in the host.

The multiple propagules concept is similar to the infectious dosage concept as the NIS impact may require one of two routes of the multiple propagules principles: either multiple deliveries of the same number of organisms OR a greater number of organisms of NIS in one delivery (i.e. density of inoculum). It is interesting to note that with greater number of propagules, a greater number of genetic varieties (i.e. genetic diversity) of the NIS species will be introduced (6). Successful NIS introduction to a new ecosystem (e.g. farmland, forest, urban setting) includes a greater genetic variety to increase the chances of successful adaption (e.g. colonization and growth) into the new ecosystem. This will also reduce the risk of a founder effect for genetic variation as well as provide genetic variation for successful colonization in other niches beyond the initial site of invasion (6).

Ruiz and Carlton (12) describe a Dose-Response relationship in the transfer of NIS to naïve niches. This relationship parallels the minimum dose of cells concept as the dose response relationship of multiple propagules can be achieved eventually via multiple discharges of numbers of organisms. Eventually, a "tipping point" or threshold is reached in the delivery of NIS organisms to achieve a successful NIS invasion and colonization. Ruiz and Carlton note that the dose response relationship will likely vary among geographic locations, time periods, habitats, vectors and taxonomic groups, yet a similar relationship and outcome will exist despite variations of invasibility or resistance to invasion from abiotic or biotic conditions in the niches (12).

All bioweapons have a requirement of a critical minimum number of organisms necessary for a successful invasion (infection). Multiple propagules can be a component used to comprehend the success of NIS invasions and hence a factor used for selection of a specific NIS as a bioweapon. One example for consideration would be *Striga* (commonly referred to as Witchweed) (13).

Striga species would be attractive as a bioweapon using the NIS approach as it produces a large number of seeds; up to 500,000 seeds per plant per year and yet are very tiny-about 0.3 millimeters long and 0.15 millimeters wide (13, 14). Various species of *Striga* can infect Corn, Sorghum, Pearl Millet, and Cowpea crops (14). If a large number of seeds were distributed in a single discharge (high density of inoculum) or in multiple discharges of lesser numbers of seeds in naïve agricultural fields, the plant parasite would have a devastating effect on agricultural productivity (aka Agricultural BW or anti-crop BW). Furthermore, *Striga* seeds can remain viable in the soil for as long as 20 years (13). Using various species of this plant parasite selectively on targeted agricultural crops, the NIS based approach could destroy or disrupt food and feedstock productivity in many countries. For example, with the high density of seeds dispersed throughout a wide area of corn fields, the resultant effect would be a serious decline in corn harvests used for feed or for biofuel production. With *Striga* using the NIS approach, this application would create a powerful bioweapon against agricultural targets in the US or other naïve niches in other countries.

3. TIME LAG/INCUBATION PERIOD ISSUES

For time lag comparison, this concept in infectious diseases is referred to as an "Incubation Period"; meaning a certain time is required for the pathogen-upon entry into the organism-to multiply and present disease symptoms (17). Many bioweapon organisms, range from hours to days to weeks prior to manifestation of disease symptoms. (9,18). For example, the incubation period for anthrax (*Bacillus anthracis*) is 1 to 5 days, for smallpox is 12 to 14 days, for plague (*Yersinia pestis*) is 2 to 8 days, for Tularemia (*Francisella tularemia*) is 1 to 14 days, and for Q Fever (*Coxiella burnetii*) is 10 to 40 days**.** This time lag is partly due to the pathogen being able to multiply in the body (e.g. rickettsia or virus inside of cells; anthrax bacteria inside of macrophages (9, 17, 18). This is not unlike the NIS Striga, the seed of this plant parasite can remain viable for 20 years until the seed comes in contact with the rhizosphere secretions of the host plant which acts as a stimulant for seed sprouting and parasitic introduction into the host plant's root structure (13).

Furthermore, the infectious organisms must be have strategies to avoid the innate or adaptive immune systems (17). Various organisms have developed strategies to avoid destruction by these components of the immune systems. For example, *Helicobacter pylori* can colonize and avoid the acidic barrier of the stomach lining (innate defense) by synthesizing and secreting ammonia to neutralize the acidity surrounding the bacteria (11) as well as altering the adaptive immune system by blocking the proliferation of antigen-dependent T- cells. This mechanism is partially accomplished by *H. pylori* secreting a vacuolating cytotoxin, VacA, which will block T-cell receptor signaling events that lead to the release of cytokines (11). Furthermore, some bioweapon pathogens are able to secrete select toxins which assist them in the disease process or in the process of spreading to other host organisms. *Bacillus anthracis* (Anthrax) toxins, which include Protective Antigen (PA), Lethal Factor (LF) and Edema Factor (EF), assist in combination or separately in the germination of anthrax spores (LF & EF-both in combination with PA); lysing macrophages resulting in the release of bacteria into the blood (LF & PA), as well as incapacitating phagocytes and the cytokines pathway (EF & PA) (19).

For infectious diseases, this means that a minimum amount of time is necessary for the pathogen to incubate in the subject (e.g. plant, animal, human) and to manifest pathological symptoms (17). For the toxin from the pathogen, the incubation (or time lag) time is necessary for the toxic effects (e.g. tissue damage, disrupted function of circulation, edema, etc.) to appear as the dosage of the toxin builds up systemically or locally.

One recent theory by Schmid-Hempel and Frank (20) helps to explain some of the variations of the pathogen dosage issue. The authors propose that organisms whose pathogenic mechanisms act locally will require a low dosage of pathogens. Those organisms that have a pathogenic mechanism that requires distance action (e.g. toxins, immune modulators, etc.) will require a larger pathogen dose to achieve infection. The authors assert that locally acting pathogen molecules require fewer cells than distantly acting pathogens whose virulence agents (e.g. toxins) diffuse easily and thus require a greater number of cells to achieve sufficient concentration of the diffusible pathogenic agents (20). The authors may in fact help tie together the incubation period/ time lag issue with the multiple propagules / minimum number of pathogen cell issue. If the infectious agent (OR NIS) requires the release of a toxin or secretion to enhance its survival and proliferation in the host (or as with NIS with a habitat), then the time lag will be greater to account for the build up to a “critical mass” of the toxin concentration to influence or enhance the survival of the pathogen or NIS. IF the pathogen or NIS does not require any toxin release or secretion to assist in its survival the incubation time (or for NIS time lag) will be comparably shorter and the cell numbers (or number of NIS propagules to achieve successive invasion) will be less.

It must be noted that NIS organisms can act to release various substances to affect their ecosystem or the organisms that would act as competitors (i.e. biotic resistance) to NIS colonization and expansion.

Although the concepts of NIS secreting products to disrupt biotic competition or alter abiotic conditions were extensively discussed in Chapter 4, it is worth mentioning several examples here as a comparison to the methods that pathogens use to evade immune defenses, competition, or alter the host organism. Reinhart and Callaway (21) discuss the various means that NIS organisms alter the soil biota. The authors review studies that found that NIS grasses doubled gross nitrification rates partly by increasing the abundance of ammonia-oxidizing bacteria in the soil. In the California grasslands, NIS species are less dependent on arbuscular mycorrhizas (AM) than native grasses. These NIS advantages or alterations can shift the nutrient availability of the soil and hence create disturbed habitats which would create a more favorable habitat for further NIS invasions (21).

Stinson et al (22) describes how Garlic Mustard (*Alliaria petiolata*), an non-mycorrhizal Eurasian biennial herb that has become an NIS in North American forests, can disrupt the inoculum potential and growth of arbuscular mycorrhizal fungi (AMF). The authors studied the anti-fungal properties of the allelopathic chemicals (glucoinolate hydrolysis products such as: allyl isothiocynate, benyl isothiocynate, and glucotropaeolin) obtained from Garlic Mustard roots. The authors found that even in undisturbed habitats, the Garlic Mustard allelopathic chemicals could inhibit mycorrhizal colonization of tree seedlings from typical northeastern temperate forests. The trees tested were Sugar Maple (*Acer saccharum*), Red Maple (*Acer Rubrum*), and White Ash (*Faxinus Americana*). The trees vary in their dependency to AMF, but the studies demonstrated that Garlic Mustard allelopathic chemicals would inhibit growth of the tree seedlings as well as virtually eliminate native AMF from soils (22). These studies demonstrates how an NIS could release substances that inhibit the habitat's soil chemistry (abiotic factor), alter AMF (biotic factor), reduce competing native plant growth (reduced competition or biotic resistance to NIS invasion) as well as alter the soil chemistry-even in a previously undisturbed habitat rendering it more favorable for further NIS invasions by the same or different species.

Several issues of time lag time for NIS must be reviewed. Kowarik (23) notes that two types of time lags occur before a rapid growth in the NIS population. These two are: the period of time between the first introduction and the first spread beyond the initial site (regardless of the success or failure of the invasion), and the period of time after the first time lag which precedes a switch to a significantly higher rate of population growth (23). Kowarik also notes that NIS that are pioneer species, favor disrupted habitats, and that cultivation or disrupted habitats favor enhanced reduction of NIS time lags (23).

Binggeli (24) discusses the three reasons for time-lags for NIS invasions is genotypic adaptions, cyclical disturbances or a combination of abiotic factors, and NIS species that have exponential growth, but are not observed until the population reaches a critical size (24). With genetic adaption, it is possible that with the greater the genetic variability within the NIS introduced, the genotypes that favor the naïve niche the best are favored and thrive; the time lag is merely the period of time that the favored genotype attains population supremacy in the native habitat and undergoes exponential growth. Crooks (6) mentions that even small genetic changes could alter the time lag of an NIS, even causing a host switch; as a one nucleotide mutation lead to a host shift with one strain of cucumber mosaic virus (6). Andow (25) describes how the rice water weevil (*Lissorhoptrus oryzae*) evolved upon entry into the United States from a native wetland grass consumer in its native Caribbean Islands to a strain that feeds on rice and later to a parthenogenic strain. Andow notes that the evolutionary steps of a new host range (feeding on rice) and reproductive strategy (parthenogenesis) lead to the rice water weevil achieving NIS success in the rice paddies of Japan and mainland Asia (25). Crooks (6) and Mack (26) note that cultivation shields the NIS from invasion failure and hence reduces the lag time of NIS invasion. Mack (26) notes that cultivation includes protection from predators, parasites, drought, frosts, etc. and can shield the NIS from environmental stochasticity (i.e. the random variation in environmental factors that influence population parameters affecting all individuals in that population)(27). Thus, with cultivation, NIS avoids some time lag effects of cyclical disturbances or abiotic conditions favorable to time lags. Daehler (28) also notes that time lags for NIS invasions can be shorter for tropical plants introduced into tropical habitats. The author asserts that reduced temperature variability and year round sunlight favor reduced time lags from introduction to becoming an invasive pest (28).

Humans (6, 26) appear to enhance NIS invasions, and it is via humans that fail to detect invasions that contribute to the final time lag variable according to Binggeli (24). For example, Daehler (28) notes that NIS history for Hawai'i may be error laden and hence may not accurately define the actual time lags of NIS invasions. Kiritani and Yamamura (29) describe some of the challenges to invasion detection which lead to large time lags. These challenges for NIS invasions of insects include the inexact time of NIS invasion, possible multiple invasions of the NIS species, and uncertainty of pathways of NIS introduction. Dray et al (30) discusses this issue of the Australian punk tree (*Melaleuca quinquenervia*) as an NIS to Florida's Everglades ecosystems. The authors research finds that human distribution of seeds and seedlings confounded the study of the determination of NIS naturalization-obscuring time lag analysis. Furthermore, the determination of the origins and invasion assist in time lag analysis of both time lag phases as described by Kowarik (23). Dray et al (30) reported difficulty in precise analysis, but based on USDA reports, local newspaper reports, and nursery catalogs, the initial lag phase ranged from ten to twenty years and was assisted by human cultivation with eventual naturalization no later than 1930. The second time lag phase was more difficult to determine, because growth and distribution of the NIS was assisted by human cultivation for forestry and landscaping applications. The authors conclude that the exponential growth of *Melaleuca* in the Everglades was not truly understood until studies in the 1990's demonstrated that this growth phase was in effect since the mid-1960's (30).

The parallel to a pathogen's incubation period is the NIS invasion time lag. Each process has parallels to how the pathogen or NIS enters the host or habitat and begins strategies to reduced resistance (e.g. immune system or biotic resistance) to its presence as well as alter its surroundings to favor further production of offspring and the spread of offspring organisms to other parts of the host or habitat.

4. EARLY DETECTION ALLOWS FOR INCREASED SUCCESS OF COUNTER STRATEGIES.

Whether it is the early diagnosis of a BW based infection or a common communicable disease, early detection can vastly improve the outcome of the patient as it can lead to rapid intervention strategies against the pathogen (17). Because of the threats to society and public health posed by BW agents, techniques are under active development to improve the speed and accuracy of the detection of BW agent (aka biothreat agents). In viewing just a sample of recent articles, the techniques range from peptide arrays (31), microarrays (32), suspension arrays (33), to Surface-Enhanced Raman Spectroscopy (35) and to techniques which can identify the impact of BW agents on host peripheral blood mononuclear cells (34). The central theme of these developments is to increase the speed as well as the accuracy of the test as well as to rule out "false positives" from hoax "mysterious powders" and contaminated samples. With this rapid and accurate diagnosis, the survival of the host (depending on the host being plant, animal, or human) is increased as strategies of vaccines, antibiotics, quarantine, containment, etc., can then be enacted.

Although quarantine, containment, eradication, and prevention are similar to NIS counterstrategies, the parallels need some review. One major concept is to prevent invasion by NIS. Andow (25) described how the previous focus on preventing NIS invasion has focused on the two ends of the invasion process: the organism in the native habitat and the habitat at risk of invasion; the author asserts that prevention needs to include the pathways by which NIS is transported to the new habitat (25). This may include review of ballast water from transoceanic shipping; transport of grains, foods, and lumber; or even the packaging containers and packing dunnage itself. Furthermore, Andow states that deliberate release of potential NIS requires risk assessment of the organism and the uncertainty of NIS invasions makes risk management difficult. If eradication efforts are compatible to vaccination and antibiotic applications post exposure, Baskin states that eradication efforts can be costly and research has indicated that beyond a certain level of colonization, eradication efforts are useless (2, pg 240), even WITH application of biocontrol measures .

Similarly, if a pathogen spreads within an organism, it may develop genetic strains that improve its virulence within the host-such as HIV (17). In NIS invasions, this can be compared to multiple introductions (multiple propagules) (36). For example, Burdon and Brown (37) observed that the invasions of Purple Loosestrife (*Lythrum salicaria*) were successful as a result of the genetic diversity obtained during multiple introductions. Novel genotypes will emerge post-invasion via selective force in the new habitat, hybridization, and genetic exchange with multiple propagules (from past or future NIS propagules releases). It is these new more invasive strains that can emerge to create colonization (similar to greater virulence in pathogens) beyond the initial habitat (36).

Ruiz and Carlton (12) describe the need for an "early-detection and rapid-response" system to detect initial invasions of NIS species and rapidly move to eradication, containment, and control. But, the challenge of NIS time lags can frustrate early detection efforts, especially if the NIS invasion takes years or decades to detect (12). Hence, the authors state that limitations exist in a possible "early detection" and "rapid response" system beyond "known NIS pests", due to limits of detection capability as well as limits of monitoring techniques and predicting the impacts of NIS with limited taxonomic and /or historical knowledge (12). These limitations will further hamper efforts for risk assessment and risk management.

One final note is that NIS invasions would appear to behave more like a chronic infection than an acute infection. That is, chronic infections have prolonged durations and often have progressively debilitating effects (12, 17). The description by Ewald of cultural vectors (38) as transmitters of pathogens parallels the vector transfer of NIS propagules, but further the long incubation times of chronic infections parallels NIS initial invasion behavior. Finally, the challenge of initial detection by the pathogen (e.g. a virus or rickettsia hiding inside cells) can parallel the challenge of early detection of NIS invasion. Hence some of the counterstrategies used for persistent infections and chronic diseases may be useful in helping to devise counterstrategies to NIS early detection or comprehending NIS behavior in naïve habitats. (SEE TABLE 1)

Therefore, although some parallels exist between the early detection of a pathogen in a host and early NIS invasion into a habitat; the problem of management and detection of actual NIS invasions can be limited. As such, it appears easier to treat an anthrax exposure than to treat an NIS invasion of Purple Loosestrife. Resources and technologies will be needed to develop early detection and rapid response techniques for NIS invasions. In light of these limitations, this may be one reason that selective application of NIS as a BW agent would be favored.

5. POPULATION DYNAMICS: THRESHOLDS FOR INVASION

Gubbins et al (39) provides a good theoretical model for the features that are characteristic of host-parasite invasions. In the paper, Gubbins uses a model based on plant-parasite interactions (here parasite is a term used generically-even though it could be used for pathogens or NIS). The model takes into account two major features of these invasions. The threshold of an invasion is based on the basic reproduction number of the parasite, R_0, which is generally defined as the average number of new infections produced when a single infective individual is introduced into a wholly susceptible host population. For a parasite to succeed, it requires $R_0 > 1$. Glass (58) describes the formula for the Reproductive Ratio (R_0). Where B is the transmission parameter (e.g. sexual contact, respiratory, food-borne, air-borne), N is the population size of susceptible hosts, and D is the duration of infectiousness. R_0 is number of "successful" contacts (secondary cases of the disease) with susceptible hosts per unit time times the length of time an individual is infectious (D) (SEE FORMULA 1).

$$R_0 = B * N * D \quad \text{(FORMULA 1)}$$

Glass (58) notes that if the R_0 is greater than 1 (that is, each primary case produces more than one secondary case), then this is an epidemic (for example: HIV has an R_0 of 11 to 12, Mumps has an R_0 of 11 to 14, and Diphtheria has an R_0 of 4 to 5.). If the R_0 is less than 1 (that is, each primary case produces less than one secondary case), then the disease will die out. If the R_0 is equal to 1 (that is, each primary case produces ONLY one secondary case), then the disease will persist as an endemic disease (58).

Furthermore, Gubbins (39) notes that the threshold host population density, N_T must be sufficient to achieve invasion and for the disease to spread beyond the initial (primary infection) infected organisms. Gubbins refers to the threshold host population density as N_T (39), whereas Glass (58) describes it as N.

Gubbins states (39) that the two features of plant-parasite interactions are the dual source of inoculums (infection from primary or externally introduced inoculums and the secondary infections from contact between susceptible and infected host tissue) as well as the host response to the infection load (SEE FIGURE 1). In cases where the parasite invasion is not probable through primary or secondary infections alone, the sum of both primary and secondary infection may allow the parasite to be able to invade. The invasion criterion for Gubbins' model states that there exists a threshold population of susceptible hosts below which the parasite is unable to invade. The model can also account for nonlinearities in the population dynamics (due to the transmission process or host response) that can create threshold densities (of either infected hosts or parasite populations) whereby the invasion cannot occur. This model has applications in not merely plant-parasite epidemiological modeling, but in other host-pathogen modeling as well (39).

The host response could affect transmission or basic parasite success, if the host response is nil to invasion, or if the adverse effects or stimulatory effects of the host response to invasion lead to the survival of the host to achieve transmission of the parasite to susceptible hosts (e.g. such as a chronic infection).

If the invasion threshold for NIS is based on the number of organisms introduced and reproduction of these organisms (R_0) as well as the abiotic and biotic factors, then the model could be considered for NIS population applications (SEE FIGURE 2). If the R_0 is increased by succeeding introductions of organisms, then the risk of NIS success into a new niche is increased (12). R_0 can be increased by multiple propagules OR high rates of offspring at the initial invasion (e.g. high rates of seeds produced, asexual reproduction of organisms as well as sexual reproduction modes) (40). These examples can parallel the primary and secondary effects of infection. The initial invasion by NIS to a niche would represent the primary infection with the NIS propagules representing the initial number of invasion organisms. These could be delivered via one large release of organisms or multiple releases of a modest number of organisms. The secondary infection would be the passage of offspring propagules from the initial NIS organisms beyond the initial site of niche introduction. The secondary infection variable would be enhanced if the organism can rapidly produce a large number of offspring at the initial site of invasion (e.g. produce large amounts of seeds, offspring). If the niche territory were compared to Host population (N_T), then the spread and expansion of NIS could be related to host population invasion variable of the Gubbins model (39).

The parallel to the Host Response would be the abiotic and biotic factors that would hinder or help the NIS propagules. In naïve niches, NIS invasions are favored in some situations as the naive niche lacks predator organisms to limit the growth and spread of NIS organisms (36, 41) (hence, this would parallel a nil host response in the Gubbins model-resulting in rapid success of inoculums in the host). Biotic and Abiotic factors are generally considered variables to NIS success by creating resistance to NIS invasion and colonization. It is known that disrupted niches can enhance the success of NIS invasions and colonization (41, 42). Even human disruptions such as road ditches and war ravaged battle fields have been found to favor NIS introduction and colonization (44). As such, disrupted niches act as blunted host responses (similar to immunocompromised patients to nosocomial pathogens) to NIS invasion thresholds. With limited abiotic factors and biotic factors, the NIS invasion can have a higher chance of success. Thus, if host response is blunted or disturbed by human or other factors, the threshold of invasion is increased. Also, agricultural sites with monoculture practices or cattle ranches with limited genetic diversity would be favorable for NIS invasions as the genetic diversity of the host organisms (e.g. corn, cattle, sheep, and wheat) is limited and thus more favorable to NIS colonization (45, 46, 47, 48). Finally, the spread of propagules in a niche that has been altered by the initial NIS organisms (e.g. soil contaminated by allelopathic chemicals of the NIS) (49, 50) can be an example of altered host response (i.e. abiotic and biotic variables reduced due to reduced competition of NIS organisms with native organisms). The altered niche would be more favorable to subsequent NIS introduction and hence expand and perhaps accelerate the spread of the NIS in the niche (51). This could lead to an "Invasion meltdown" by multiple NIS invasions of differing species (59).

In summary, the Gubbins model (39) could be applied to NIS invasions to help describe the process and numbers necessary to attain successful invasions. If NIS is introduced in high numbers either in one invasion OR lower number of propagules introduced in multiple introduced (e.g. multiple discharges of NIS laden ship ballast water (52), then the R_0 will be high and favor NIS success (i.e. invasion threshold). Also, if the niche has been disrupted by human or other activity or if the niche genetic diversity is reduced, then the NIS introduction will be favored (i.e. reduction in host responses). Gubbins notes that primary and secondary infection can be summated to achieve the threshold criteria (39). Hence, with NIS invasions, a strategy of multiple exposures (e.g. multiple propagules) is likely to favor NIS success from a population dynamics approach. In examining the population dynamics of plant-parasite interactions, another parallel between host-parasite epidemiology and NIS invasions can be made. These parallels will continue to be reviewed in time and it is conceivable that as better models develop, the predictability of NIS invasions and countermeasures for such invasions will be improved. It is also conceivable that as the models develop, they can be used to fine tune techniques to use NIS as a biological weapon.

6. BACKFIRE OF A WEAPON (OR NIS INTRODUCTION!)

In consideration of the potential use of NIS as a biological weapon, the potential for the backfire of a biological weapon has to be considered. The concept of a backfire is when the weapon used by an aggressor on an adversary comes back to damage or injure the aggressors' forces, native territory or civilian population; this includes injury to the actual agents manufacturing or dispersing the biological weapons. In previous uses of Biological Weapons, the potential for a backfire has been feared, but the concerns are not without merit.

Alibek (53) describes how he determined that during World War II, the Soviets used Tularemia against invading German Panzer troops during the summer 1942 invasion near the Volga and Stalingrad. Unfortunately, the dispersal of the bacterial agent led to a severe Tularemia outbreak in Soviet military forces as well as the resident civilian population (53, pg 30-31). Alibek also describes the infection and death of a research scientist while developing a highly virulent and lethal strain of Marburg virus (strain U) as a biological weapon (53, pg 126-131, 54). Koenig (55) hints that it was possible that during World War I, German agents using Glanders to infect US horses and mules which would be used in European battlefields, may also have been infected with the disease. Alibek (53, pg. 70-86) as well as Mangold and Goldberg (56) describe the accident at the biological arms production facility at Sverdlovsk in which a missing aerosol filter led to the escape and dispersal of weaponized anthrax spores and hence contaminating the local population. This "accident" lead to civilian deaths ranging from 60 to 600 and to military personnel deaths ranging from 250 to 300. The accident also triggered an official U.S. investigation of Soviet cheating on the 1972 Biological Weapons Convention Treaty (56).

Many weapons that are aerosolized can return from the direction of the defender forces back upon the attacker force by a mere shift of the wind. One classic example of this event is described by Croddy et al (57) with the use of Chlorine gas (chemical warfare) by German forces during World War I. The German directed attack using Chlorine gas on French entrenched forces was fouled up by shifting winds that returned the Chlorine gas back onto German entrenched forces (57).

The potential release of NIS as a BW could have backfire effects if the NIS undergoes any genetic enhancements to fitness which result in increased virulence or fitness in its original native habitat. This is not out of the realm of possibility. Braiser (60) describes research in which the NIS fungi causing Dutch Elm Disease, *Ophiostoma ulmi*, through interspecific gene transfer became a more virulent pathogen upon re-entry to its native Europe leading to a greater pandemic of an originally endemic pathogen. Braiser notes that interspecific gene flow is creating hybrids rapidly and leading to the rapid emergence of new and modified fungal plant pathogens (60). Brasier et al (61) further describes analysis of interspecific hybridization of a *Phytophthora* species resulting in a newly evolved hybrid that was pathogenic to alder trees, whereas the parent species, *Phytophthora cambivora*, was not pathogenic to alder trees. Man in't Veld, et al (64) describe the development of a hybrid *Phytophthora* strain on *Spathiphyllum* and *Primula* plants in the Netherlands that appears to have evolved from a cross of an endemic species and an introduced species. Using Isozyme analysis and Random Amplified Polymorphic DNA analysis, the hybrid was an interspecific hybrid of *Phytophthora nicotianae* (NOTE: the introduced species) and *Phytophthora cactorum*. The authors note that this interspecific hybridization can be a means to extend the host range or develop new hosts for the species as well as become a means for rapid development of new species (64).

Spiers (62) describes how hybrids of an introduced species of the rust fungus *Melampsora*, were able to infect poplar trees in New Zealand, whereas the parent fungal strains could not infect the rust resistant poplar tree strains. Newcombe et al (63) describes how two fungal leaf rusts of *Melampsora* formed a hybrid rust fungus, *Melampsora xcolumbiana*, which infected hybrid poplars in the Pacific Northwest. Newcombe et al notes that when host ranges of both fungal parent rust species overlap, the hybrids can act as a genetic bridge to transfer pathogenicity traits from one rust species to another (63).

As stated in previous chapters, some forms of fungal plant pathogens were developed for BW (53, 56). Palm and Rossman (45) comments on reviewing the above studies with a warning that hybrid fungal pathogens can develop and become more virulent or attack previously resistant hosts. The authors state that the mechanism is due in part to the repeated exposure of new fungal germplasm from NIS (such as the multiple propagules approach!).

If NIS were used in a multiple exposure approach for BW, it is possible, depending on the NIS species (and endemic species present) as well as the potential for interspecific gene transfer, for the development of new strains of NIS species with the potential to backfire upon the aggressor disseminating the original NIS strain in BW format.

7. SUMMARY

In the research to investigate the possibility of BW application of NIS, a comparison of the pathogen infection of a host to the invasion of NIS to a naïve habitat can be developed. The concept of multiple propagules is one key to successful NIS invasion parallels the host-pathogen concept of minimum dosage of cells necessary to achieve an infection. The concept of time lags for the period from NIS invasion to colonization to exponential growth parallels the incubation period of pathogens (including BW agents) in the host body. In both instances, the time lag/incubation period is a temporal factor necessary to overcome innate and adaptive immune defenses (for the pathogen) or abiotic and biotic resistance in the naïve habitat (for the NIS species).

The Gubbins population dynamics model (39) of plant-parasite interaction can be compared to and applied to NIS invasions. The parallels of the host-parasite epidemiology and NIS invasions can be useful in comprehending NIS behavior and assistive in fine tuning BW applications of NIS.

The backfire concerns of BW applications of NIS must be taken seriously. NIS organisms have demonstrated the capability to increase their invasibility, virulence, and host shift potential due to interspecific gene transfer, mutation, and repeated exposure to new germplasms from exotic and/or endemic species. Although initial studies have demonstrated this phenomena for fungal NIS, it is possible future studies will detect these events occurring in other NIS species across the various taxonomies (i.e. from bacteria to mammals). In each event of NIS BW usage, the aggressor developing NIS weapon applications must seriously research if interspecific gene transfer or hybridization could create a "backfire event" with the new NIS returning to devastate the aggressors' own habitats and ecosystems.

Finally, one brief follow up of a previously mentioned comparison. Mack (16) referred to the analogy of epidemics caused by parasites by comparing it to NIS invasions. Yet, unlike acute infections, NIS invasions act more similar to chronic infectious diseases as described by Ewald (38). Furthermore, similar to "smoldering infections" that appear over time, NIS time lags are long and difficult to detect until (usually) colonization or exponential growth is underway. As the organism of a chronic infectious disease develops, it may undergo genetic changes to enhance its virulence and infect other hosts. NIS may undergo genetic changes or selective pressures to achieve strains with greater fitness to the new habitat as well as capabilities to spread beyond the initial invasion habitat. The pathogen in a chronic infectious disease must have a means to spread its offspring to infect other hosts (including use of vectors). Likewise, NIS offspring must have select fitness to spread beyond the initial invasion habitat to new habitats. The overall result of a chronic infectious disease is progressively debilitating effect on the host. The overall result of NIS on habitats is a progressive weakness of native species (some can become extinct); the NIS may overrun the habitat, and the habitat's abiotic and biotic components are altered in such a way to make further NIS (of other species) invasions possible. (SEE TABLE 1)

In essence, one analogy to NIS invasion could be a comparison to a chronic infectious disease. Hence, the BW applications of NIS could lead to long term serious effects on target habitats (e.g. urban, rural, forest, agricultural). Understanding this comparison could lead to better model development of NIS invasions as well as better counterstrategies for NIS invasions or against BW applications of NIS.

TABLE 1-COMPARISON OF NIS INVASIONS AND CHRONIC INFECTIONS	
CHRONIC INFECTION-ALSO CALLED "STEALTH INFECTION" (EWALD)	**NIS INVASION**
SLOW DEVELOPMENT TIME- CAN RANGE FROM MONTHS TO DECADES-PERIOD OF TIME MAY NOT DEMONSTRATE ANY EVIDENCE OF SYMPTOMS. EXAMPLES: HIV, SHINGLES, SUBACUTE SCLEROSISNG PANENCEPHALITIS, CYTOMEGALOVIRUS, HERPES SIMPLEX VIRUS, HEPATITIS C, ADULT T-CELL LEUKEMIA (HTVL-1) (17, 38)	TIME LAGS CAN RANGE FROM MONTHS TO DECADES; DURING PERIOD OF TIME NO EVIDENCE OF NIS PRESENCE OR HABITAT DAMAGE MAY BE PRESENT. (23, 24, 28)
LONG PERIOD OF DEBILIATING ILLNESS OF HOST. EXAMPLES: HELICOBACTER PYLORI, HEPATITIS B & C, HIV (17, 38)	-HABITAT DAMAGE MAY BE OCCUR OVER A LONG PERIOD FO TIME AND NOT DETECTED UNTIL NIS SPECIES ACHIEVES "CRITICAL MASS" NUMBERS, AS SLOW PROGRESSIVE DAMAGE ACCUMULATES (21, 24, 28, 30). -ECOSYSTEM MAY DEMONSTRATE DAMAGE, EVEN AFTER INVASION IS ENDED (21, 22).
-PURPOSE OF LONG TIME PERIOD FOR INFECTION: TO MAXIMIZIZE SPREAD OF PATHOGEN (OFFSPRING) TO OTHER HOSTS. EXAMPLES: HELICOBACTER PYLORI, HEPATITIS B & C, HIV (17, 38)	-SPREAD BEYOND INITIAL SITE OF INVASION TO OTHER HABITATS (2, 30). -ALTERED NICHE MAY ACCELERATE SPREAD OF NIS (51). -MULTIPLE PROPAGULES ENHANCE SPREAD OF NIS (4, 5, 6, 12)
-GENETIC DIVERSITY (NEW STRAINS, VARIATIONS IN VIRULENCE FACTORS) OCCURS DURING INFECTION. EXAMPLES: INFLUENZA, HIV, NOSOCOMIAL *Escherichia coli* (17, 38)	-DURING NIS INVASION: GENETIC FITNESS AND NEW STRAINS DEVELOPED, ESPECIALLY WITH RECOMBINATION OF NEW PROPAGULES FROM SUBSEQUENT RELEASES. SELECTIVE FORCES OF HABITAT CREATE NIS STRAINS WITH BETTER FITNESS FOR NEW HABITAT AND CAPACITY TO SPREAD BEYOND INITIAL SITE OF INVASION. (6, 25, 26, 37)
-IN IMMUNOCOMPROMISED PATIENTS, A GREATER SPREAD OF PATHOGENS WITHIN THE PATIENT AND TO OTHER SUSPECTIBLE PATIENTS. EXAMPLES: SPREAD OF Kaposi's Sarcoma, *Pneumocystis carinii* (Pneumocystosis), *Toxoplasma gondii* (Toxoplasmosis) (17, 38)	-EVIDENCE HABITATS INVADED BY ONE NIS CAN BECOME EASIER TO HAVE OTHER NIS SPECIES INVADE LATER. ENHANCEMENT OF NIS INVASIBILITY IN DISTURBED HABITATS AND MONOCULTURE SITES (MONOCULTURE DEMONSTRATED REDUCED GENETIC DIVERSITY). (23, 25, 41, 42, 44)
-CULTURAL VECTOR TRANSFER. EWALD- INCLUDES DOCTORS, NURSES, CARE-TAKERS, MOVE PATHOGENS FROM IMMOBILIZED HOSTS TO SUSPECTIBLE HOSTS. EXAMPLES: CHOLERA, DYSENTERY, *Clostridium difficile,* NOSOCOMIAL *Escherichia coli* (38)	-VECTORS FOR TRANSFER- HUMAN SHIPPING, DUNNAGE, BALLAST WATER, DELIBERATE HUMAN TRANSPORT OF NIS FOR SPORT OR PETS, VECTORS THAT MOVE NIS FROM NATIVE HABITATS TO NAÏVE HABITATS AND SPREAD NIS INVASIONS. (6, 25, 26, 30)

FIGURE 1- MODEL OF Gubbins (39) thresholds for invasion.

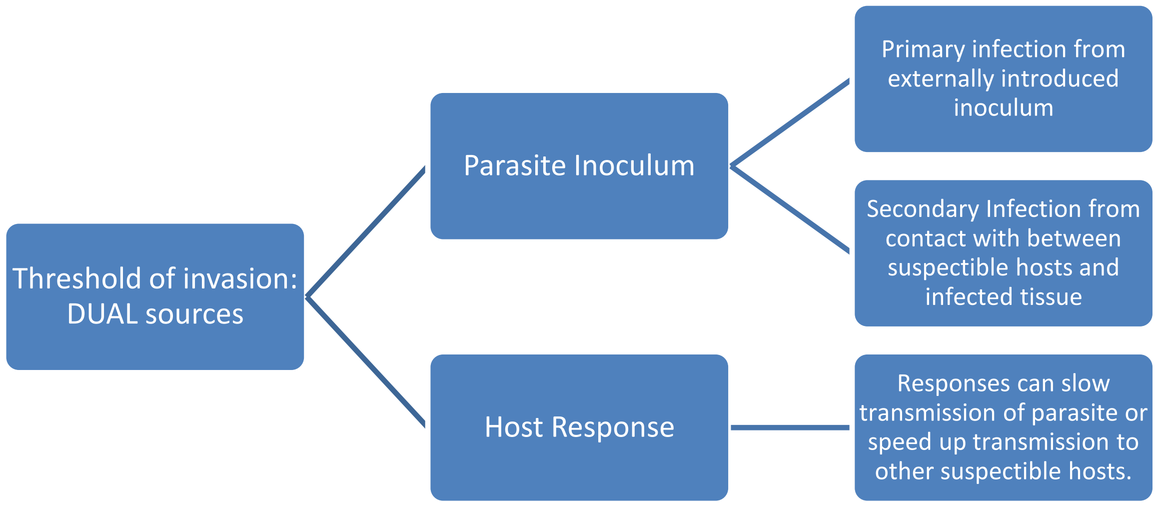
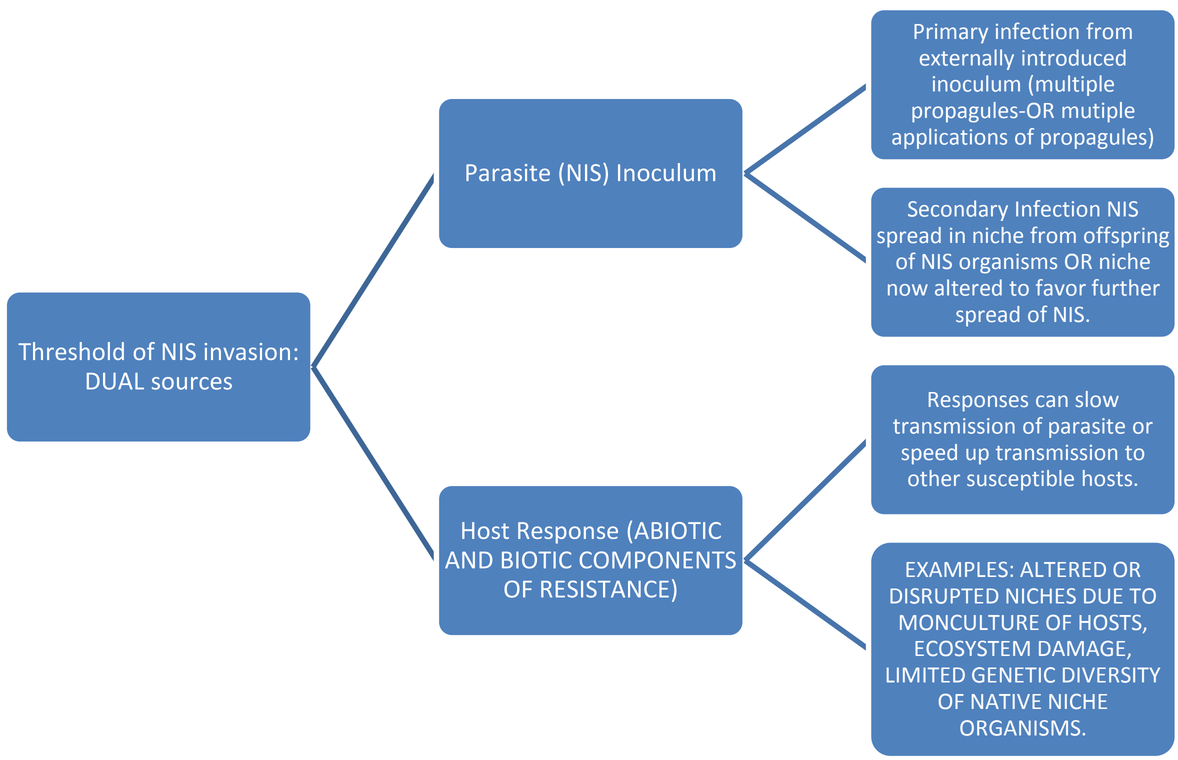


FIGURE 2-Parallels of Gubbins Model to NIS invasions



9. CITATIONS:

1- Colautti, Robert I., and Hugh J. MacIsaac. "A neutral terminology to define 'invasive' species." Diversity and Distribution 10 (2004): 135-141.

2- Baskin, Yvonne. A plague of rats and rubber vines: The growing threat of species invasions. Washington, DC: Island Press, 2002.

3- Colautti, Robert, I., Niimi, Arthur, J., et al. "Spatial and Temporal Analysis of Transoceanic Shipping Vectors to the Great Lakes." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 227-246.

4- Verling, Emma, and et al. "Supply-side invasion ecology: characterizing propagule pressure in coastal ecosystems." Proceedings of the Royal Society B 272 (2005): 1249-1257.

5- Reichard, Sarah Hayden, and Hamilton, Clement W. "Predicting Invasions of Woody Plants Introduced into North America." *Conservation Biology*. 11.1 (2002): 193-203.

6- Crooks, Jeffrey A. "Lag Times and Exotic Species: the Ecology and Management of Biological Invasions in Slow-motion." *Ecoscience*. 12.3 (2005): 316-329.

7- Fuller, Pam L.. "Freshwater Aquatic Vertebrate Introductions in the United States: Pattern and Pathways." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 123-151.

8- Marty, A. M., "History of the Development and Use of Biological Weapons." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 421-434.

9- Franz, D.R., et al. "Clinical Recognition and Management of Patients Exposed to Biological Warfare Agents." Clinics in Laboratory Medicine: Laboratory Aspects of Biowarfare, Vol. 21, No 3. ED. Marty, A. M., Philadelphia, PA: W.B. Saunders, 2001, 435-474.

10- Donnenberg, Michael S. "Pathogenic Strategies of Enteric Bacteria." *Nature*. 406. (2000): 768-774.

11- Merrell, D. Scott, and Stanley Falkow. "Frontal and Stealth Attack Strategies in Microbial Pathogenesis." *Nature*. 430. (2004): 250-256.

12- Ruiz, Gregory, M., Carlton, James T. "Invasion Vectors: A Conceptual Framework for Management." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 459-504.

13- Nour J., Press M.C., Stewart G.R., and Tuohy, J.M.' Africa in the grip of witchweed". *New Scientist.* 109. (1986): 44–48.

14- Mohamed, K. I., Papes, M., Williams, R., Benz, B.W., and Peterson, A.T. "Global Invasive Potential of 10 Parasitic Witchweeds and Related Orobanchaceae." *Ambio*. 35.6 (2006): 281-288.

15- Yershov, A.L., B.S. Jordan, C.H. Guymon, and M.A. Dubick. "Relationship between the Inoculum dose of Streptococcus pneumoniae and Pneumonia onset in a Rabbit Model." *European Respiratory Journal*. 25.4 (2005): 693-700.

16- Mack, Richard N., Daniel Simberloff, and W. Mark Lonsdale, et al. "Biotic Invasions: Causes, Epidemiology, Global Consequences and Control." *Issues in Ecology*. 5. (2000): 1-20.

17- Nester, Eugene, Denise Anderson, Nancy Pearsall, and Martha Nester. *Microbiology*. 4th Ed. New York, NY: McGraw-Hill Science/Engineering/Math, 2004. 463.

18- O'Brien, Karen K., Mark L. Higdon, and Jaime J. Halverson. "Recognition and management of Bioterrorism Infections." *American Family Physician*. 67.9 (2003): 1927-1934.

19- Moayeri, Mahtab, and Stephen H. Leppla. "The Roles of Anthrax Toxin in Pathogenesis." *Current Opinion in Microbiology*. 7. (2004): 19-24.

20- Schmid-Hempel, Paul and Steven A. Frank. "Pathogenesis, Virulence, and Infective Dose." PLOS Pathogens 3(10) Oct 2007 e147. 04 Dec 2007.

21- Reinhart, Kurt O., and Ragan M. Callaway. "Soil Biota and Invasive Plants." *New Phytologist*. 170. (2006): 445-457.

22- Stinson, Kristina A., Stuart A. Campbell, and Jeff R. et al Powell. "Invasive Plant Suppresses the Growth of Native Tree Seedlings by Disrupting Belowground Mutalisms." *PLOS Biology*. 4.5 (2006): e140. DOI: 10.1371/journal.pbio.0040140.

23- Kowarik, Ingo. "Time Lags in Biological invasions with Regard to the Success and Failure of Alien Species." *Plant Invasions-General Aspects and Special Problems*. ED. P. Pysek, K. Prach, M. Rejmanek, and M. Wade. Amsterdam, The Netherlands: SPB Academic Publishing, 1995, 15-38.

24- Binggeli, P. 2001. Time-Lags between introduction, establishment and rapid spread of introduced environmental weeds. Proceedings of the Third International Weed Science Congress; 2000 June 6-11; Foz do Iguassu, Brazil, Manuscript number 238, 17 p., CD-ROM. Available from: International Weed Science Society, Oxford, MS, USA.

25- Andow, David A. "Pathways-Based Risk Assessment of Exotic Species Invasions." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 439-455.

26- Mack, Richard N. "Cultivation Fosters Plant Naturalization by Reducing Environmental Stochasticity." *Biological Invasions*. 2. (2000): 111-122.

27- "Environmental Stochasticity definition." *Glossary E, Genetics and Genomics Glossary*. U.S. Geological Survey, 08-Aug-2008 . Web. 30 Jul 2010. <http://biology.usgs.gov/genetics_genomics/glossary_e.html>.

28- Daehler, Curtis C. "Short Lag Times for Invasive Tropical Plants: Evidence from Experimental Plantings in Hawai'i." *PLoS ONE*. 4.2 (2009): e4462. Doi:10.1371/journal.pone0004462.

29- Kiritani, Keizi, Yamamura, Kohji, "Exotic Insects and Their Pathways for Invasion." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 44-67.

30- Dray, F. Allen, Bradley C. Bennett, and Ted D. Center. "Invasion History of Melaleuca quinquenervia (Cav.) S.T. Blake in Florida." *Castanea*. 71.3 (2006): 210-225.

31- Taitt, C.R., North, S.H., and Kulagina N.V.. "Antimicrobial Peptide Arrays for Detection of Inactivated Biothreat Agents." *Peptide Microarrays: Methods in Molecular Biology*. Ed. Marina Cretich and Marcella Chiari. Clifton, NJ: Humana Press, 2009, 233-255.

32- Leski, Tomasz A., Baochin Lin, and Anthony P. Malanoski, ET AL. "Testing and validation of High Density Resequencing Microarray for Broad Range Biothreat Agents Detection." *PLoS ONE*. 4.8 (2009): e6569, doi:10.1371/journal.pone.0006569.

33- Wang, Jing, and Yu Yang, ET AL. "Simultaneous detection of five biothreat agents in powder samples by a multiplexed suspension array." *Immunopharmacology and Immunotoxicology*. 31.3 (2009): 417-427.

34- Das, Rina, and Rasha Hammamieh, ET AL. "Early indicators of exposure to biological threat agents using host gene profiles in peripheral blood mononuclear cells." *BMC Infectious Diseases*. 8. (2008): 104 doi:10.1186/1471-2334-8-104.

35- Golightly, Rebecca S., William E. Doering, and Michael J. Natan. "Surface-Enhanced Raman Spectroscopy and Homeland Security: A Perfect Match?." *ACSNano*. 3.10 (2009): 2859-2869.

36- *Predicting Invasions of Nonindigenous Plants and Plants Pests*. National Research Council. Washington, DC: National Academy Press, 2002.

37- Brown, A.H.D. and J.J. Burdon, 1987." Mating systems and colonization success in plants. " *Colonization Succession and Stability*. Ed. A.J. Gray, M.J. Crawley, and P.J. Edwards, Oxford, UK: Blackwell Scientific Publications,1987, 115-131.

38- Ewald, Paul W. Plague Time: The New Germ Theory of Disease. New York, NY: Anchor Books, 2002.

39- Gubbins, Simon, and Gilligan, Christopher A., Kleczkowski, Adam, "Population Dynamics of Plant-Parasite Interactions: Thresholds for Invasion." *Theoretical Population Biology*. 57. (2000): 219-233.

40- Meinesz, Alexandre, and Translated by Daniel Simberloff. Killer Algae. Chicago: University of Chicago Press, 1999.

41- Hansen, Malin J., and Clevenger, Anthony P. "The influence of disturbance and habitat on the presence of non-native plant species along transport corridors." *Biological Conservation*. 125.2 (2005): 249-259.

42- Mitchell, C.E., and A.G. Power. "Release of Invasive Plants from Fungal and Viral Pathogens." *Nature*. 421. (2003): 625-627.

43- Mack, Michelle C., and D'Antonio, Carla M., "Impacts of biological invasions on disturbance regimes." *Trends in Ecology & Evolution*. 13.5 (1998): 195-198.

44- Mack, Richard N. "Global Plant Dispersal, Naturalization, and Invasion: Pathways, Modes, and Circumstances." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 3-30.

45- Palm, Mary E., Rossman, Amy Y., "Invasion Pathways of Terrestrial Plant-Inhabiting Fungi." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 31-43.

46- Parker, Henry S., Agricultural Bioterrorism: A Federal Strategy to Meet the Threat. 1st ed. Honolulu, HI: University Press of the Pacific, 2004.

47- Tatum, L. A. "The Southern Corn Leaf Blight Epidemic." *Science*. 171. (1971): 1113-1116.

48- Dudley, Joseph P., and Michael H. Woodford. "Bioweapons, Biodiversity, and Ecocide: Potential Effects of Biological Weapons on Biological Diversity." *BioScience*. 52.7 (2002): 582-592.

49- Callaway, Ragan, and W.M. Ridenour. "Novel weapons: Invasive Success and the Evolution of Increased Competitive Ability." *Frontiers in Ecology and the Environment*. 2.8 (2004): 436-443.

50- Callaway, R.M., D. Cipollini, and et al. "Novel Weapons: Invasive Plant Suppresses Fungal Mutualists in America but not in its native Europe." *Ecology*. 89. 4 (2008): 1043-1055.

51- Bulleri, F., J.F. Bruno, and L. Benedetti-Cecchi. "Beyond Competition: Incorporating Postive Interactions between Species to Predict Ecosystem Invasibility." *PLOS Biology*. 6.6 (2008): e162. doi:10.1371/journal.pbio.0060162.

52- Colautti, Robert, I., Niimi, Arthur, J., et al. "Spatial and Temporal Analysis of Transoceanic Shipping Vectors to the Great Lakes." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 227-246.

53- Alibek, Ken, and with Stephen Handelman. Biohazard: The chilling true story of the largest covert biological weapons program in the world-told by the man who ran it. New York: Random House, 1999.

54- Hart, John. "The Soviet Biological Weapons Program." *Deadly Cultures: Biological Weapons Since 1945*. Ed. Mark Wheelis, Lajos Rozsa, and Malcolm Dando. Cambridge, MA: Harvard University Press, 2006, 132-156.

55- Koenig, Robert. The Fourth Horseman: One man's mission to wage the great war in America. New York: Public Affairs, 2006.

56- Mangold, Tom, and Goldberg, Jeff. Plague Wars: The Terrifying Reality of Biological Warfare. New York: St. Martin's Press, 1999.

57- Croddy, Eric, Perez-Armendariz, C., and Hart, J.. Chemical and Biological Warfare: A comprehensive survey for the concerned citizen. New York: Copernicus Books, 2002.

58- Glass, Gregory. " Measuring Disease Dynamics in Populations: Characterizing the Likelihood of Control." Open Source Ware-Johns Hopkins Bloomberg School of Public Health course. 2007. Johns Hopkins School of Public Health. 30 Sep 2007 < http://www.jhsph.edu/preparedness/training/online/disease_measure.html>.

59- Simberloff, Daniel, and Betsy Von Holle. "Positive interactions of nonindigenous species: invasional meltdown?." *Biological Invasions*. 1. (1999): 21-32.

60-Brasier, Clive M. "Rapid Evolution of Introduced Plant Pathogens via Interspecific Hybridization." *BioScience*. 51.2 (2001): 123-133.

61-Brasier, C.M., D.E.L. Cooke, and J.M. Duncan. "Origin of a new *Phytophthora* pathogen though interspecific hybridization." *Proceedings of the National Academy of Science USA*. 96. (1999): 5878-5883.

62-Spiers, A.G. "*Melampsora* and *Marssonia* pathogens of poplars and willows in New Zealand." *European Journal of Forest Pathology*. 28. (1998): 233-240.

63-Newcombe, G., et al. "*Melampsora xcolumbiana*, a natural hybrid of *M. medusae* and *M. occidentalis*." *Mycology Research*. 104.3 (2000): 261-274.

64-Man in 't Veld, Willem A., Veenbaas-Rijks, Wil J., et al. "Natural Hybrids of *Phytophthora nicotianae* and *Phytophthora cactorum* Demonstrated by Isozyme Analysis and Random Amplified Polymorphic DNA." *Phytopathology*. 88.9 (1998): 922-929.

CHAPTER 6. NIS BIOLOGICAL WEAPON DETERMINATION: METHODS TO DETERMINE NIS WEAPONIZATION AND EFFICIENCY

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1. INTRODUCTION

The weaponization of NIS requires knowledge of which NIS species would make a good candidate for a weapon and this knowledge includes some prediction of its efficiency as a weapon. Therefore, this chapter examines the concepts and tools that would help in the decision making process for which species of NIS could be a biological weapon as well as where such a weapon would be successful or useful (target selection). It must also be noted that "usefulness" as a weapon does not necessarily means that the NIS invasion was successful (as demonstrated by the paper by Mack and D'Antonio (1) which discussed the after effects of NIS invasions on damaging the ecosystem). Therefore, a failed invasion may cause ecological damage or even psychological damage to the targeted society; as would be the intent for a bioterrorist (1).

Therefore, if the objective of the BW attack is to damage the niche (or entire ecosystem), then successful BW damage may not necessarily require successful invasion and colonization- just damage to the ecosystem- or even the appearance of "contamination" by entry of the NIS to the target niche. It must also be remembered that ecosystems include urban habitats, agricultural fields, estuaries, forests, or other habitats where food, biofuels, or other resources are obtained from a society aside of the aesthetic or biophilic value of the niche (2).

The key questions for weaponization of NIS are: what is the organism and where can it be applied? Since Peterson (11) notes that one limitation of prediction of a species invasion is the evolutionary change of ecological niche parameters; a third question may require the consideration of what amount of ecological shifting could or can occur in the niche. Shifts must be considered not merely by quantity, but with regard to the amount of time by which the shift has occurred or could occur. If the shift in parameters is minor, this may not affect NIS invasion success. IF the shift in parameters is extreme, then these changes may negatively or positively affect NIS invasion success. Also, it is conceivable that NIS invasion success can alter some niche parameters and these factors may require review in subsequent modeling. It is possible that an invasion meltdown (27), as a possible BW strategy, could be incorporated (that is, succeeding NIS introduction of species one after another to achieve niche collapse, disease outbreaks, or area denial (29) via the BW attacks).

2. HISTORY

The history of NIS has been previously discussed in Chapter 4. Using the history of any NIS as a guide for possible weaponization has some value. Nevertheless, the value or practice of NIS history is limited. For example, the merits of the history of Kudzu (*Pueraria lobata*) are that once introduced into the Southern United States, the plant exhibited such rapid growth so as to smother many other plant species in a particular niche (3). In a study by Kolar and Lodge (4), reviewing various NIS invasion papers, the reviewers found that history can be used for some species. The authors found that the probability of NIS success in plants increases if the species (as well as the family or genus) has a history of invasion (4). A study by the National Research Council (5) found that history of an NIS can be a strong indicator of invasibility and hence it is used in evaluating risk factors for the introduction of the potential species into naïve habitats.

But, the history of a species may be no indicator of its effect in other ecosystems on other continents. This is due in part to the limitations of the biogeographical factors that exist in those potential target areas. These factors are not merely sunlight, temperature, and yearly rainfall, but may include other abiotic factors such as soil chemistry as well as biotic factors (e.g. predators, parasites, pathogens, etc.).

Also, the history of many species is still unknown to its effects beyond its documented known site of invasion and there is less known of invasion failures (6). Ruiz and Carlton (6) discuss some of the limitations of history as a predictive tool. In part, the history of NIS success is limited in some parts of the world and due to limited resources to detect high-impact invasions or the precise timing of invasions; a historical record is limited for many species (6).

The advantages of a history approach is that the known effects on the biotic and abiotic factors in naïve niches may provide some information on its invasive effects, once the NIS is delivered to a target site that is similar to other invaded niches.

The disadvantages of using NIS history is that the previous invasion history is known by environmental and government agencies, but in many cases this information comes well past the post-colonization phase when eradication or biocontrol steps are warranted (6). These agencies and scientists may have to obtain the limited resources (e.g. funding, manpower, public support, education, communication) to implement counterstrategies to eradicate the invasion or colonization. Furthermore, since the history of invasion by the NIS is known, that capacity to detect early infestations by wildlife specialists and government environmental specialists is much greater (IF the wildlife personnel are trained to know what to look for!). If early detection occurs, the effectiveness of NIS as a BW will be reduced as the NIS may be eradicated before serious damage to the target niche can occur. Finally, various agencies (e.g. United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS), New Zealand's Ministry of Agriculture and Forestry and Environmental Risk Management Authority (ERMA)) have developed interdiction and border control policies to prevent the importation of NIS (7, 8). Although skilled bioterrorists and determined agents of rouge states can out maneuver these policies and practices (e.g. smuggling); the skilled border agents will have been trained in the detection of KNOWN NIS (that is, NIS with prior history of being invasive organisms). These interdiction policies will further reduce the probabilities of BW success with historically known NIS organisms.

3. ONE STEP APPROACH

Beyond the history of any potential NIS species, Peterson et al (9) describes the use of Ecological Niche Modeling (ENM) using primary event data. ENM (also referred to as Environmental Niche Modeling) is the process of using computer algorithms to develop predictive maps of species distribution in geographic space based on mathematical representation of known or inferred distributions in ecological niches. This process utilizes data that summarizes the spatial distribution of environmental parameters (e.g. soil chemistry, altitude, mean annual temperature, mean sunlight, etc.) essential for the model (12, 30). The process of converting primary observations of occurrence into a collection of spatially continuous information has various approaches. The eventual outcome is to take the data of known events and convert it into a biogeographical map of the presence or absence of a species. This map could be used to provide predictive capability of NIS invasive success in naive niches.

Peterson et al (9) describes the one-step approach developed by Hollander et al (10). Hollander et al (10) developed the spatial arrangement of species distribution (called a biodiversity data set) based on known occurrence points of the species, the Orange-Throated Whiptail bird (*Cnemidophorus hyperythrus*), to map out the range limits of the species. Peterson et al (9) states that one-step (i.e. one-step as it focuses on mere geographical distribution based on spatial arrangement of known occurrence points) models are convenient as they are based on known data of geographical distributions and are often less expensive computationally (9). But, Peterson (9, 11) notes that the limitation of this ENM mapping is that the one-step approach does not distinguish between ecological space and geographic space. The mapping requires the assumption of uniform sampling and can be subject to error due to species diversity errors and the failure to note that species distributions may be due to complex interactions of ecological and historical variables (9).

As a mapping approach, one-step mapping offers some insight into species distributions, but offers no real predictive value for NIS invasibility or advantage for NIS weaponization requirements.

4. TWO STEP MODEL

Peterson et al (9, 12) describes the Two-Step modeling approach as a means to directly tie mapping to species biology. The modeling is referred to as "Two Step" because the first step develops the model niche in **ecological space** and the second step projects the model on to the **geographic space** (11). As the ecological factors of species distribution, the ecological niche (e.g. temperature, precipitation, sunlight, etc.) are developed, they are then modeled to hypothesize the environmental conditions that are capable of maintaining a stable population. This model can then be projected back onto the geographic map to render a prediction of the native range OR in the case of NIS invasions, predict the range of naïve niches susceptible to NIS invasibility (11, 12). Joseph Grinnell developed the concept of the species ecological niche such that the ecological conditions limit the species' distribution potential, while at the same time maintain the population without immigration of individuals from other areas (11, 38). The maintenance of long-term stability of an ecological niche is an underlying assumption for the success of Ecological Niche Mapping and for the success of predictive models of NIS invasions (11). Ecological niches provide a set of possible factors under which a species is able to invade and succeed in a naïve niche (12).

Before a model can be developed, biodiversity data must be obtained in the species' autochthonous niche to develop the initial data of the native range. This biodiversity information is obtained from scientific collections that identify a particular species at a particular place (9, 12). Primary data (i.e. occurrences observed and documented by scientific specimens and locality information) are favored over secondary data that usually consists of range maps, ecological summaries, and species accounts. This is because the point of occurrence data (which includes species density or patchiness in spatial distribution of species) that is critical for ecological niche model programming is lacking in the secondary data. This is due to the characteristics of secondary data that include publication lag times from the time of observation and the subjectivity of mapping which includes unsampled areas within maps (9). With primary data being the preferred form of biodiversity data, the problem arises over obtaining the data. Much primary data is not computerized and may include older (e.g. decades or older) data (9). This challenge has been off-set by the development of a database called, The Species Analyst, developed by The North American Biodiversity Information Network of the Commission for Environmental Cooperation (Montreal, Canada) and the National Science Foundation (US). This database is using an ANSI/Z39.50 standard of information retrieval as well as XML language for the searching and retrieval of information from the various global biological collections connected to the Internet (9, 12, and 13).

With the means to obtain primary point occurrence data for a particular species, the development of two-step models can progress (14). Although other models exist, this paper will focus on two promising models, BIOCLIM and GARP.

5. MODEL-BIOCLIM

BIOCLIM, (short for BIOCLIMATIC) (18) was one of the earlier approaches to modeling niches which involved counting species occurrences into categories, trimming marginal ranges of distribution, and considering the niches as a set of ranges of bioclimatic indices (e.g. mean temperature, minimum temperature, annual temperature, annual precipitation, etc.) (15). Peterson et al (9) states that BIOCLIM is easy to implement, but suffers from reduced efficacy when excessive bioclimatic variables are included. These excessive variables can lead to over-fitting of the model and a misrepresentation of species potential ranges. This is hinted by Nix (15) who first used BIOCLIM with only 12 climatic indices for his landmark 1986 study on the biogeographic distribution of Australian Elapid snakes. Furthermore, a study by Doran and Olsen (16) found BIOCLIM to be less effective for highly mobile species such as the case with the seasonable distribution of the eastern grass owl (*Tyto capensis*) in Australia (16). Earlier versions of BIOCLIM consisted of 35 climatic parameters throughout the species' known range.

Beaumont et al (17) suggested that a reduction of values to those responsive to a specific species and actual size distributions of the species will enhance the predictive distributions of BIOCLIM. In a study that compared the predictive distributions of 25 Australian butterfly species, the researchers compared BIOCLIM using 35 bioclimatic parameters (full set), a "customized set" based on the biology of the species in question, and a "generalized set" of 8 parameters that commonly appeared to influence the distributions of the 25 butterfly species. The results indicated that the 35 bioclimatic parameters lead to an "over-fitting" of distribution (narrower potential distribution) in all parameter sets; but the "customized set" resulted in the least over-fitting of the predictive model (17). The researchers suggest that BIOCLIM would be more successful as a predictive distribution tools (hence minimize errors) if the selected parameters are directed more to the species of interest. Jeschke and Strayer (14) note that although bioclimatic models (including BIOCLIM) can be successful in mapping present-day species distributions; it is limited in forecasting NIS invasive ranges or species migrations due to climatic change. These limitations the authors note are because the models follow several unreasonable assumptions: constancy of species genotype and phenotype over time; ignoring effects of biotic interactions over time; and unlimited species dispersal (14).

BIOCLIM can be useful as a predictive tool of species diversity and hence for NIS invasiveness. But, as the predictability range of distribution can be limited by uncertainty within certain variables or if certain variables have more impact on that specific species, this form of modeling has limitations for NIS prediction of BW usefulness. It is important to keep in mind that BIOCLIM is deterministic in nature; that is, based on a single decision rule (yes or no) and hence its error in predicting species distribution can be further enhanced by shifts in climate (11). Finally, as Peterson noted (25), BIOCLIM suffers generally from high rates of commission error (aka Overprediction); this may account for need of trimming marginal ranges, but nevertheless this accounts for a degree of uncertainty in predictive mapping.

6. MODEL-GARP

GARP (Genetic Algorithm for Rule-set Prediction) is a genetic algorithm devised by Stockwell to address the problem of species distribution modeling (19, 20, 21). The goal of GARP was to develop a genetic tool with reliable performance on a range of data to examine a range of potential species-ecosystem relationships (21). Stockwell notes that a secondary goal was to provide a reasonable explanation for the systems' predictions (19). The genetic algorithm (GA) approach could provide robustness through the use of multiple models and apply the "generate and test" approach to evaluating alternative models. Furthermore, the process allowed for interpretation of members of GA population as rules which would allow the prediction of the system to be explained (19). To achieve a GA, a class of algorithms was developed to reflect the concept of evolution by natural selection; that is, the solutions to biological problems are evolved in a stochastic iterative fashion similar to the way that species evolve (19). A GA is devised by creating a set of potential solutions to a problem and iteratively modifying the set until an optimal solution can be devised; in essence, GA's are an adaptive search technique (32). As individual algorithms are used (e.g. logistic regression, Bioclimatic rules, etc.) to produce component "rules" in a broader rule set (rule superset), then portions of species distributions can be determined (i.e. species presence inside versus outside of the niche or geographic boundary) based on different rules of the algorithms. Peterson notes, in essence, GARP is a superset of other ENM approaches and should always perform BETTER than any of the other forms of ENM (9).

As noted previously, models developed by GARP are composed of rules, IF-THEN relationships as the rules are developed, tested, and selected. Three criteria for estimating the utility of rules are applied: statistical significance, predictive accuracy, and usefulness. After the rules are produced by GA, they are calibrated for accuracy to an independent test map (previously devised based on museum or other point of occurrence data). This strategy applies the rules to the problem of predicting the outcome at each point on the test map (19). The strategy for rule selection is to adopt those rules which predict the geographical location of the species (or provide an estimate of the probability of presence at each point) with the highest expected accuracy and maximizing the total accuracy of the GA (19) (i.e. convergence).

Examples of successful GARP applications include the Greater Glider (*Petauroides volans*)-a gliding possum in forest regions of South eastern Australia (19), the North American invasion of the NIS aquatic plant *Hydrilla verticillana* (11), the Spiny Pocket Mouse (*Heteromys anomalus-Heteromyidae*) in Columbia and Venezuela (22), the Passerine bird (*Carpodacus mexicanus-Fringillidae*) in Western North America and South Mexico (22), the Wood Thrush (*Hylocichla mustelina*) in Maine (9), invasion of the Fire Ant (*Solenopsis invicta*) in North America (24), global invasion potential of Witchweed (*Striga*) and Broomrape (*Orobanche*) (26, 31), and 34 species of North American passerine birds (25). Many studies included examining the invasive potential of NIS species. Peterson (11) in reviewing a variety of GARP based studies on plants and animals, freshwater and terrestrial, vertebrates and invertebrates; concluded that the GARP predictivity of the geographic course of NIS invasions has been "excellent" (11). The author notes that the predictability of GARP demonstrates that species follow ecological rules that can be assembled based on their native distributional niches wherever they exist in the world (11).

These studies further demonstrated the usefulness and accuracy of GARP for mapping species distributions in native niches as well as the usefulness and accuracy of mapping NIS invasions in naïve niches. Also, Stockwell and Noble (21) noted that the advantage of GARP over BIOCLIM was that GARP's robust modeling system was much more stable against random perturbances of data. Examples of perturbances of data include climatic change, changes in abiotic factors, and shifts in population density (21). Since GARP is a rule based modeling system, perturbations act on single rules, not the rule set; hence the rule set undergoes only a partial change, BUT not a complete restructuring of the rule set as would occur in a decision tree induction system (such as used in BIOCLIM) (21). Stockwell notes that the consensus approach of multiple models compensates for problems in one model and provides good results on most occasions (33). Peterson (25) notes that GARP testing has demonstrated insensitivity to dimensionality of environmental data which is one of the shortcomings of BIOCLIM (25, 28).

Nevertheless, it should be noted that several limitations exist with GARP. But, with proper foresight and planning, many of these limitations can be surmounted and a robust map of species distribution (or NIS invasion) can be obtained. Peterson (11) notes that one limitation of using GARP is that it is computation intensive. The author states that a "typical" analysis (e.g. 40 to 50 base environmental coverages, 1000 to 10,000 iterations) often requires 5 to 10 minutes of CPU time at 1 GHz processing speed. BUT, an "ideal" analysis requires 100 or more base environmental coverages and 10,000 to 100,000 iterations which can absorb HOURS of computing time per model (11). Although the author notes that considerable computational capacity is necessary for model development for a single species, it must be noted that work station processor (CPU) speeds have improved since the 2003 publication of this paper (11).

One other technique worth mentioning that could overcome this challenge is the networking of ordinary desktop PC's to create supercomputer processing capabilities. Hargrove et al (34) describe the use of discarded PC's (using Intel 486 and Pentium microprocessors) linked together by Ethernet cards, a Linux operating system, and parallel programming to create "Stone Souper Computer"-a supercomputer with the theoretical peak performance of about 1.2 gigaflops (i.e. billion floating point operations per second) (34). The system-called Beowolf-created a map of ecoregions of the continental United States consisting of 25 environmental characteristics for 7.8 million one square kilometer cells and then reduced the information into 1000 ecoregions (35). With this computing power and the relative inexpensive costs for development, the applications of GARP could be easily expanded for NIS environmental or BW applications. BUT, the present "desktop version of GARP" is precluded from this approach as the desktop version is WINDOWS based (not capable of a LINUX operating system) and can not be applied to parallel processing, except where each species examined was assigned to a single PC processing unit (37). Of course, this does not rule out the development of a modified version of GARP for such "Stone Souper Computer" applications.

Another limitation factor for GARP accuracy is the availability of point occurrence data (11). As described earlier, any predictive environmental niche model is only as good as the point occurrence data that it is based on. If the data is not accurate for the grid cell in the environmental data; then the accuracy will be reduced in the predicted distribution (20). Stockwell (19) states that overprediction is common in models using only climate based data. The author notes that species distributions can change due to changes in habitat in a select area or that species are no longer present in some areas (i.e. local extinction) or in areas that are geographically separated and passage between the areas is rare for the species (or in the cases of human induced changes-migration is blocked) (11, 19).

Errors due to point occurrence data can be divided into two classes: omission error or under prediction (aka false negatives) and commission errors or overprediction (aka false positives). NOTE: both of these can be reduced as the point occurrence data is more precise (i.e. the" fine grain detail" or reduction of the size of the pixel on the grid map as compared to the "course grain" of detail in the map), the more accurate the GARP results.

Stockwell and Peters (20) explain that errors can be due to missing values. The authors note that sampling bias in ecology is due to the dependence on presence-only data (point occurrence data). Sampling bias can introduce unwanted patterns in the data. Most museum databases record where species were collected, but NO information exists on where species did

NOT occur. This can represent a sampling bias for a particular set of the dependent value (data of species presence only). Background data (where the species was not present) is absent. GARP provides a solution for this by generating pseudo-absence data called "background", which is based on selecting points at random from the geographical space. The data set for GARP may consist of present, background, and IF true absent record data is present, it will also be included. This strategy helps to reduce errors in GARP analysis (20).

BUT, several other factors must be brought up that can limit a species distribution to a smaller area. Peterson (9, 11) notes these four key factors that reduce a species distribution to smaller areas (hence render GARP predicted mapping with some errors): Limited dispersal (limits to species encountering suitable distributional areas, especially if the areas are disjunctive from the present species distributional area); Speciation (allopatric speciation can create sister species in suitable distributional areas previously inhabited by an ancestral species); Extinction (species population may have previously existed in a site, only to become extinct and leave behind an uninhabited but suitable distribution area); and Competition (interspecific competition can to some degree limit species geographic distribution as well as create absences of species in suitable distributed areas) (9, 11). This is not always observed until after the review of the GARP analysis. Furthermore, it should be noted that human interference can alter the limited dispersal by human intervention of migration patterns or habitat destruction or even species reintroduction (2-see pages 104-105, 276-277, 281-282).

To counter errors, Peterson and Cohoon (28) note that by jackknifing and bootstrapping (i.e. statistical resampling methods) geographic information coverages, select coverages for the rule set development stand out and hence will decrease the omission and commission errors. Stockwell and Peterson (36) demonstrate that in obtaining sample size of species distributions-a law of diminishing return eventually arises (36). The authors found that using GARP in general could create coarse models with 90% accuracy with ten sample points and achieved near maximal at 50 data points, whereas a fine model would have a lower increase in accuracy with a maximum accuracy achieved at about 100 data points. The authors noted that accuracy began to decrease with increasing sample sizes beyond the afore mentioned amounts (hence, the concept of "diminishing return"!). Thus, sample size must be considered in the use of GARP as a predictive tool. Peterson and Vieglais (12) noted that by using a "test model" with selective test data to assess the robustness and accuracy of GARP-using 4 to 8 environmental data sets and 10 to 30 occurrence points-the GARP models developed were more than 90% correctly predicted. Thus, in the use of GARP, the point of occurrence data need not be very high, but it must be valid.

In summary, GARP appears to provide a strong predictive model for NIS invasions, while at the same time minimizing errors of omission and commission. As such, GARP modeling would be favored –along with sufficient point of occurrence data of the candidate NIS organism-as a tool for NIS BW development.

7. GARP STUDIES WITH POTENTIAL BW AGENTS

The concept of using GARP in BW selection and development goes beyond mere speculation. Several BW approaches are presently supported by successful GARP analysis of potential BW agents or vectors for such agents. The agents include Marburg virus (a hemorrhagic fever), Dengue Fever (a painful and debilitating disease also called "breakbone fever"), and Monkeypox (an Orthopoxvirus similar to smallpox, but less contagious and less lethal in humans).

Peterson discusses how ENM can be very useful in investigating the potential for spread of disease by examining the vectors, pathogens, or hosts for the diseases (39). One example is the GARP analysis which predicted the spatial dynamics of the vector insects and eventual human cases of Dengue Fever in Mexico (40). The study demonstrated the potential for forecasting the disease transmission risk by the predicting of the spatiotemporal dynamics of disease vector species. Two important comments to mention with this disease as it relates to BW. A number of authors cited the development of Dengue Fever as a BW agent by culturing infected mosquitoes at Camp Detrick for the US BW program in the 1950's (41, 42, 43, 44) as well as research into using Dengue Fever as a BW agent by the French BW program in the 1960's (45). Furthermore, Lockwood noted that during World War II, Japanese General Ishii Shiro realized that insect vectors would be advantageous- for BW- as an operational weapon as they protected the pathogen from environmental degradation, provided the conditions to reproduce, and carried the pathogen agent directly to the human enemy (44, pg 88). Therefore, present day GARP analysis could be useful in determining the outbreak of a Dengue Fever based BW attack by the prediction of the spatiotemporal dynamics of a covert release of infected mosquitoes to a target area (i.e. niche).

Marburg virus is a hemorrhagic viral disease in which the vector or reservoir for the virus is not clearly understood (46). Yet, GARP analysis has demonstrated the geographic potential for outbreaks of this disease based on previous outbreaks in the Africa continent, including the potential for outbreaks in countries where the disease is not presently known to exist (46, 47). Since the disease has a high mortality rate, it was favored for BW research and eventual weaponization by the former Soviet Union (42, 48, 49) as well as a pathogen of BW research for South Africa (41). Presently, although confined to a select set of African nations, without a clear understanding of the natural reservoir population for the virus, GARP could provide predictive modeling for BW applications of this pathogen in naïve niches or populations (including human).

Finally, although Monkeypox is an Orthopoxvirus similar to the Smallpox virus, it is less infectious and less lethal than Smallpox; monkeypox vector GARP studies provide an insight into the potential threat of this virus as a BW agent. The reasoning behind this statement is due to the extensive research done by the former Soviet Union's biological weapons program both in the weaponization of the virus and the research into genetically engineering the virus to enhance its virulence and mortality (48, 51). Monkeypox is also listed in the US Military Field Manual of potential biological warfare agents (52).

One recent incident of human Monkeypox in 2003, was epidemiologically tracked to exotic pets-African Giant Pouched Rats (*Cricetomys*)- imported from Africa containing the zoonosis. The West African viral strain of Monkeypox spread to prairie dogs (*Cynomys* species) and eventually to humans caused by the rats which were sold in pet stores in the mid-west United States (50, 53, 54). Reed et al (54) describe the transmission pathways and timetable of the outbreak with human cases appearing to occur from contact (e.g. bite, cage cleaning, etc.) with the prairie dogs. In a follow up study by Croft et al (53), the researchers question whether some human cases with no contact with the prairie dogs occurred via viral exposure in the veterinary facilities from aerosolization of respiratory secretions or environmental exposure to viral laden animal urine or feces. Also, Croft et al (53) and the Reed et all (54) could not rule out human-to-human transmission in two cases during the outbreak, but evidence is uncertain due to the lack of personal protective equipment use among the veterinary staff. Finally, Frey and Belshe (55) speculate that as immunity to smallpox wanes in the general population and as further popularity of exotic pets rises in society, therefore the risk of human disease from animal orthopoxviruses may increase.

Peterson et al (50) describe a GARP study examining the invasive potential of the African giant pouched rat, *Cricetomys* (both *C. gambianus* and *C. emini*). Both species are carriers of a variety of pathogens, including the MonkeyPox virus. Since the reported Monkeypox-*Cricetomys* incident in 2003, Peterson et al researched using GARP, what could be the invasive potential of these rats in North America (50). One species, *C. gambianus* was found to have a broad potential invasiveness across the Southeastern United States. Based on the GARP study, a monkeypox infected vector (or worse, vector carrying a genetically engineered monkeypox virus) such as *Cricetomys* could spread the virus in a covert but deliberate BW attack, which could result in the virus infecting and killing native fauna or humans as well as becoming endemic in select areas of the United States. If *Cricetomys* became an NIS, it could certainly be the reservoir for Monkeypox in the United States resulting in disease and death for years afterwards. Witmer et al (56) notes that the Gambian Giant Pouched Rats (*Cricetomys gambianus*) is already a threatening invasive species on a Florida island, Grassy Key. The USDA's Wildlife Services has initiated an eradication and detection effort on Grassy Key, but the trapping of the sparse population of these rats has proven challenging. Witmer et al (57) reports some success in the development of attractants which help in the trapping and eradication efforts. Still, if this species were to attain landfall in the US, as reported by the Peterson study, it could become a disruptive invasive species as well as a serious reservoir for Monkeypox as well as other diseases which would be transmissible to humans, livestock, and wildlife (56).

8. NEW SPECIES-NEW NIS SPECIES-NEW BW AGENT?

With new species being discovered each year, it is noteworthy to consider the possibility that some of these newly discovered species could become potential NIS BW agents. As the Catalogue of Life site (58) cited that about 2/3 of the all of the planet's species have been catalogued, many more species are being added yearly to the encyclopedias of biodiversity across the globe. Conservation International (59) with its rapid assessment program (60), along with the Census of Marine Life (61) and many other organizations have performed biological surveys across the globe to discover and understand new species in various niches. The taxonomic research publications as well as the accompanying genetic, ecological, climatic, and sometimes geospatial data is then collected and presented in various online archives such as the Catalogue of Life (62) (a collaboration of Species 2000 and the Integrated Taxonomic Information System) and the Encyclopedia of Life (63). This data along with new genetic bar coding performed by the Consortium for the Barcode of Life (64) for all species provides an extensive database for the determination of newly discovered species as well as family and genus relationships of known species with new species.

Yet, as these organizations and catalog compile new species data, this data can be used for future GARP studies for the potential invasive capabilities of these species. Furthermore, if the new species exhibits family or genus relativeness to known NIS species, then the risk of NIS potential is enhanced. If the new species exhibits "pioneer species traits" (e.g. capacity to colonize in initially unsuitable or adverse niches of soil or climate, rapid maturity, rapid production of many offspring, etc.) (2, 65), this may also indicate invasiveness potential. Some pioneer species have become NIS in naïve environments (e.g. rats) (3). Yet, as global biodiversity surveys continue, data continues to be complied on the newly discovered species' ecological niche and geographical space characteristics. As this data is complied, the potential for new candidates of NIS BW will also expand.

9. SUMMARY

The weaponization of an NIS requires information on whether the candidate species would make a good NIS for the targeted site. But for that to occur, information on the organism and its NIS capabilities must be determined. In this chapter, the invasiveness of the species and hence its potential for weaponization were considered using various approaches. While no one system was without limitations or disadvantages, the evolution of invasiveness capability determination can be demonstrated in this chapter's analysis.

The first mode of invasiveness analysis is the history of the NIS. Using the NIS history of the species as a guide for potential BW applications has merit, but it is limited to known species with known NIS capabilities in known naïve niches (6). Thus, using this species in BW may well invite early detection or rapid elimination by trained border agents or environmental agency personnel prior to successful onset of NIS BW damage.

The next approach, the one step model, was originated by Hollander et al (10). This model was based on mere geographical distribution information based on known point of occurrence data of the species (10). Although less complex computationally, the one step model fails to distinguish ecological space from geographic space and hence is subject to errors in determination of species distributions. Therefore, the NIS predictive value for the one step model tool is rendered nil.

The two step model is an approach that connects mapping to the species biology. The two steps model develops the species model niche in ecological space first, and then projects that model onto geographic space (11). After the two step model is completed, the model can then be used to predict the range of naïve niches susceptible to NIS invasion (11, 12). It must be noted that the two step model is sensitive to the primary data of the species native niche, not secondary data such as range maps or ecological summaries (9). The primary data is most accurate when it is obtained from documented observations accompanied with locality data as well as species density and detailed spatial distributions of the species (9). Although other two step modeling programs exist, this paper focused on two promising models, BIOCLIM and GARP.

BIOCLIM (short for BIOCLIMATIC), first used by Nix (15), was an early approach to niche modeling by counting species occurrences into categories, trimming ranges of distribution, and considering niches as a set of bioclimatic index ranges (e.g. minimum temperature, annual precipitation, etc.) (15). Although BIOCLIM can be useful as a predictive tool for species diversity as well as NIS invasiveness, it suffers errors in prediction due to climatic changes, species migrations, "over-fitting" of distributions, and commission errors (11, 14, 16, and 25). The key limitation to BIOCLIM is that it is based on a single decision rule (yes-no) and hence shifts in climate increase error and mapping uncertainty (11).

GARP (Genetic Algorithm for Rule-set Prediction) is a genetic algorithm devised by Stockwell (19, 20, and 21). A genetic algorithm (GA) is an adaptive search technique using a class of algorithms (e.g. logistic regression, BIOCLIM, etc.) to develop solutions to biological problems in a stochastic iterative fashion (similar to the evolution of a species). GARP becomes a superset of environmental niche models which apply a "generate and test" approach until an optimal solution to the initial biological problem is devised (9, 19, and 32). As the model developed by GARP is composed of IF-THEN rules, the rules are developed, tested, and selected to a problem to predict the outcome of each point on a test map. The final outcome is to provide a GA that is capable of mapping the candidate species with the highest expected accuracy (19). GARP studies have been successful in a variety of species mapping studies-e.g. vertebrates and invertebrates (11). Yet GARP has limitations based on initial mapping data accuracy; changes in climate, abiotic factors, population density; large computational iteration demands; and sensitivity to point occurrence data, especially from sampling bias (11, 21). Nevertheless, GARP has been a strong predictive modeling tool for NIS invasions (11, 24, 26, and 31) as well as for potential BW agents such as Marburg virus or the vectors of Dengue Fever and Monkeypox (39, 40, 46, 47, and 50).

Finally, in reviewing the BW potential for NIS, consideration must be given to newly identified species for NIS potential and hence their potential as an agent of NIS BW. With biodiversity surveys occurring across the globe, both on land and in the oceans (60, 61); new species from all taxa are being discovered and characterized (i.e. genetically, ecologically, climatically, geographically, etc.). This data provides the means for GARP analysis of the NIS potential for these organisms and hence the BW potential for any new species identified as having NIS potential. Aside of a GARP analysis, new species may also suggest NIS potential if the species is related (by family or genus) to known NIS or if they exhibit "pioneer species" traits.

10-CITATIONS:

1-Mack, Michelle C., and D'Antonio, Carla M., "Impacts of biological invasions on disturbance regimes." *Trends in Ecology & Evolution*. 13.5 (1998): 195-198.

2- Cunningham, William P., and Barbara Woodworth Saigo. *Environmental Science: A Global Concern*. 4th ed. Dubuque, IA: William C. Brown, 1997. 6, 7, 31 32, 87, 124.

3- Baskin, Yvonne. <u>A plague of rats and rubber vines: The growing threat of species invasions</u>. Washington, DC: Island Press, 2002.

4- Kolar, Cynthia S., and David M. Lodge. "Progress in invasion biology: predicting invaders." *TRENDS in Ecology & Evolution*. 16.4 (2001): 199-205.

5- *Predicting Invasions of Nonindigenous Plants and Plants Pests*. National Research Council. Washington, DC: National Academy Press, 2002.

6- Ruiz, Gregory, M., Carlton, James T. "Invasion Vectors: A Conceptual Framework for Management." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 459-504.

7- Cavey, Joseph F. "Mitigating Introduction of Invasive Plants Pests in the United States." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 350-361.

8- Hayden, Barbara J. Whyte, Carolyn F. "Invasive Species Management in New Zealand." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 270-291.

9- Peterson, A. Townsend, Stockwell, David R.B., and Kluza, David A.. "Distribution Prediction Based on Ecological Niche Modeling of Primary Occurrence Data." *Predicting Species*

Occurrences: Issues of Accuracy and Scale. Ed. J.M. Scott et al. Washington, DC: Island Press, 2002. 617-623.

10- Hollander, Allan D., Davis, Frank W., Stoms, David M.. "Hierarchical representations of species distributions using maps, images and sighting data." *Mapping the Diversity of Nature*. Ed. Ronald I. Miller. London: Chapman & Hall, 1994. 71-88.

11- Peterson, A. Townsend. "Predicting the Geography of Species Invasions via Ecological Niche Modeling." *Quarterly Review of Biology*. 78,4 (2003): 419-433.

12- Peterson, A. Townsend, and Vieglais, David A., "Predicting Species Invasions using Ecological Niche Modeling: New Approaches from Bioinformatics Attack a Pressing Problem." *BioScience*. 51.5 (2001): 363-371.

13- Cover, Robin. "The Species Analyst Project." *Cover Pages*. Robin Cover, 17 Feb 2001. Web. 29 Aug 2010. <http://xml.coverpages.org/speciesAnalyst.html>.

14- Jeschke, Jonathan M., Strayer, David L., "Usefulness of Bioclimatic Models for Studying Climate Change and Invasive Species." *The Year in Ecology and Conservation Biology, Vol. 1134*. Ed. Richard S. Ostfeld and William H. Schlesinger. New York: New York Academy of Sciences, June 2008.1-24.

15- Nix, Henry A.. "Biogeographic analysis of Australian elapid snakes." *Atlas of Elapid Snakes* . Ed. R. Longmore. Canberra: Australian Flora and Fauna Series, No. 7, Australian Government Publishing Service, 1986. 4-15.

16- Doran, Bruce, and Penny Olsen. 2001. "Customizing BIOCLIM to investigate spatial and temporal variations in highly mobile species." *6th International Conference in GeoComputing*. Pullar, University of Queensland. Brisbane, Australia.

17- Beaumont, Linda J., Lesley Hughes, and Michael Poulsen. "Predicting species distributions: use of climatic parameters in BIOCLIM and its impact on predictions of species' current and future distributions." *Ecological Modeling*. 186. (2005): 250-269.

18- "BIOCLIM." *Publications*. The Fenner School of Environment and Society:The Australian National University , 14-Aug-2007. Web. 29 Aug 2010. <http://fennerschool.anu.edu.au/publications/software/anuclim/doc/bioclim.html>.

19- Stockwell, David R.B.. "Genetic Algorithms II: Species Distribution Modeling." *Machine Learning Methods for Ecological Applications*. Ed. A.H. Fielding. Boston: Kluwer Academic Publishers, 1999. 123-144.

20- Stockwell, David, and David Peters. "The GARP modeling system: problems and solutions to automated spatial prediction." *International Journal of Geographical Information Science*. 13.2 (1999): 143-158.

21- Stockwell, David R.B., and Ian R. Noble. "Induction of sets of rules from animal distribution data: A robust and informative method of data analysis." *Mathematics and Computers in Simulation*. 33. (1992): 385-390.

22- Anderson, Robert P., Daniel Lew, and A. Townsend Peterson. "Evaluating predictive models of species' distributions: criteria for selecting optimal models." *Ecological Modeling*. 162. (2003): 211-232.

23- Ricciardi, Anthony, William W.M. Steiner, Richard N. Mack, and Daniel Simberloff. "Toward a Global Information System for Invasive Species." *BioScience*. 50.3 (2000): 239-244.

24- Peterson, A.T., and Y. Nakazawa. "Environmental data sets matter in ecological niche modeling: an example with *Solenopsis invicta* and *Solenopsis richteri*." *Global Ecology and Biogeography*. 17. (2008): 135-144.

25- Peterson, A. Townsend. "Predicting Species' Geographic Distributions Based on Ecological Niche Modeling." *The Condor*. 103. (2001): 599-605.

26- Mohamed, Kamal I., and Papes, Monica, ET AL. "Global Invasive Potential of 10 Parasitic Witchweeds and Related *Orobanchaceae*." *Ambio*. 35.6 (2006): 281-288.

27- Simberloff, Daniel, and Betsy Von Holle. "Positive interactions of nonindigenous species: invasional meltdown?." *Biological Invasions*. 1. (1999): 21-32.

28- Peterson, A. Townsend, and Kevin P. Cohoon. "Sensitivity of distributional prediction algorithms to geographic data completeness." *Ecological Modeling*. 117. (1999): 159-164.

29- "Area denial weapons ." The Free Dictionary. Farlex, Inc., 2010. Web. <http://encyclopedia.thefreedictionary.com/Area+denial+weapon>.

30- "Environmental niche modelling." *SensAgent*. Memodata Corporation, 2009. Web. <http://dictionary.sensagent.com/Environmental_niche_modelling/en-en/>.

31- Mohamed, K. I., J. F. Bolin, L. J. Musselman, and A. T. Peterson. , "Genetic diversity in Striga and implications for control and future distributions. In " *International Symposium on Integrating New Technologies for Striga Control: Towards Ending the Witch-hunt", Addis Ababa, Ethiopia, November 5-11, 2006*. Ed. J. Gressel and G. Ejeta. Singapore: World Scientific Publishing, 2006. Pages 71-84.

32- De Jong, Kenneth. "Learning with Genetic Algorithms: An Overview." *Machine Learning*. 3. (1988): 121-138.

33- Stockwell, David R.B. "Advances in Ecological Niche Modeling ." Ecological Modeling for NISA Applied Sciences Workshop . National Aeronautics and Space Administration (NISA) and the Center for Health Applications of Aerospace Related Technologies (CHAART) . Asilomar

Conference Center, Monterey, California, Monterey, California. 30 March - 1 April 2005. Lecture, <http://geo.arc.NISa.gov/sge/health/ecoworkshop/present/Stockwell.pdf> .

34- Hargrove, William W., Forrest M. Hoffman, and Thomas Sterling. "The Do-It-Yourself Supercomputer." *Scientific American*. Aug 2001: 72-79.

35- Hargrove, W.W., and F.M. Hoffman. “Using multivariate clustering to characterize ecoregion borders.” *Computers in Science and Engineering.* 1(4) (1999): 18-25.

36- Stockwell, David R.B., and A. Townsend Peterson. "Effects of sample size on accuracy of species distribution models." *Ecological Modeling*. 148. (2002): 1-13.

37-Scachetti-Pereira , R. "Desktop GARP." *The University of Kansas Center for Research*. The University of Kansas Natural History Museum and Biodiversity Research Center, 25 February 2002. Web. 15 Oct 2010. <http://nhm.ku.edu/desktopgarp/index.html>.

38- Grinnell, Joseph. "Field Tests of Theories Concerning Distributional Control." *American Naturalist*. 51.602 (Feb 1917) (1917): 115-128.

39-Peterson AT. Ecologic niche modeling and spatial patterns of disease transmission. *Emerging Infectious Dieases* [serial on the Internet]. 2006 Dec [Oct 15, 2010]. Available from http://www.cdc.gov/ncidod/EID/vol12no12/06-0373.htm

40- Peterson, A. Townsend, Carmen Martinez-Campos, Yoshinori Nakazawa, and Enrique Martinez-Meyer. "Time-specific ecological niche modeling predicts spatial dynamics of vector insects and human dengue cases." *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 99. (2005): 647-655.

41- Harris, Robert, and Jeremy Paxman. *A higher form of killing: The secret history of chemical and biological warfare.* Revised edition. New York: Random House, 2002.

42- Regis, Ed. *The Biology of Doom: The history of America's secret germ warfare project*. New York: Henry Holy and Company, 1999.

43- Moon, John Ellis van Courtland,. "The US Biological Weapons Program." Deadly Cultures: Biological Weapons Since 1945. Ed. Mark Wheelis, Lajos Rozsa, and Malcolm Dando. Cambridge, MA: Harvard University Press, 2006, 9-46.

44- Lockwood, Jeffrey A. Six-Legged Soldiers: Using Insects as Weapons of War. New York: Oxford University Press, 2009. 197.

45- Lepick, Olivier. "The French Biological Weapons Program." Deadly Cultures: Biological Weapons Since 1945. Ed. Mark Wheelis, Lajos Rozsa, and Malcolm Dando. Cambridge, MA: Harvard University Press, 2006, 108-131.

46- Peterson, A. Townsend, John T. Bauer, and James N. Mills. "Ecological and Geographic Distribution of Filovirus Disease." *Emerging Infectious Diseases*. 10.1 (2004): 40-47.

47- Peterson, A. Townsend, R. Ryan Lash, Darin S. Carroll, and Karl M. Johnson. "Geographic potential for outbreaks of Marburg hemorrhagic fever." *American Journal of Tropical Medicine and Hygiene*. 75.1 (2006): 9-15.

48- Alibek, Ken, and with Stephen Handelman. Biohazard: The chilling true story of the largest covert biological weapons program in the world-told by the man who ran it. New York: Random House, 1999.

49- Hart, John. "The Soviet Biological Weapons Program." Deadly Cultures: Biological Weapons Since 1945. Ed. Mark Wheelis, Lajos Rozsa, and Malcolm Dando.

50- Peterson, A. T., M. Papeş, M. G. Reynolds, N. D. Perry, B. Hanson, R. L. Regnery, C. L. Hutson, I. K. Damon, and D. S. Carroll., . "Native-Range Ecology and Invasive potential of *Cricetomys* rats in North America." *Journal of Mammalogy*. 87. (2006): 427-432.

51- Croddy, Eric, Perez-Armendariz, C., and Hart, J.. <u>Chemical and Biological Warfare: A comprehensive survey for the concerned citizen.</u> New York: Copernicus Books, 2002. Cambridge, MA: Harvard University Press, 2006, 132-156.

52- <u>Potential Military Chemical/Biological Agents and Compounds</u>. Field Manual FM 3-11.9. Wentzeville, Missouri: Eximdyne, 2005. IV-19-20.

53- Croft, Donita R., and Sotir, Mark J. et al. "Occupational Risks during a Monkeypox Outbreak, Wisconsin, 2003." *Emerging Infectious Diseases*. 13.8 (2007): 1150-1157.

54-Reed, Kurt D., and Melski, John W. et al. "The Detection of Monkeypox in Humans in the Western Hemisphere." *New England Journal of Medicine*. 350.4 (2004): 342-50.

55-Frey, Sharon E., and Robert B. Belshe. "Poxvirus Zoonoses-Putting Pocks into Context." *The New England Journal of Medicine*. 350.4 (2004): 324-327.

56-Witmer, Gary W., and Bourassa, Jean B., et al. "The Path to Eradication of the Gambian Giant Pouched Rat in Florida." *Managing Vertebrate Invasive Species: Proceedings of an International Symposium*. EDS. Witmer, G.W., Pitt, W.C., Fagerstone, K.A.: Fort Collins, CO, USDA/APHIS/WS, National Wildlife Research Center. 2007. 305-311.

57-Witmer, Gary W., Nathan P. Snow, and Patrick W. Burke. "Potential Attractants for Detecting and Removing Invading Gambian Giant Pouched Rats (Cricetomys gambianus)." *Pest Management Science*. 66. (2010): 412-416.

58-"About the Catalogue of Life." *Catalogue of Life: 2011 Annual Checklist*. N.p., 2011. Web. 28 Jun 2011. <http://www.catalogueoflife.org/info/about>.

59- C*onservation International*. Conservation International Foundation , 2011. Web. 28 Jun 2011. <http://www.conservation.org/Pages/default.aspx>.

60- "The Rapid Assessment Program is celebrating its 20th Anniversary ." *Conservation International*. Conservation International., 2011. Web. 28 Jun 2011. <http://www.conservation.org/how/field_work/discoveries/Pages/expeditions_discovery.aspx>.

61- "About the Census." *Census of Marine Life*. Census of Marine Life, 2010. Web. 28 Jun 2011. <http://www.coml.org/about-census>.

62-Bisby F.A., Roskov Y.R., Orrell T.M., Nicolson D., Paglinawan L.E., Bailly N., Kirk P.M., Bourgoin T., Baillargeon G., Ouvrard D., eds (2011). Species 2000 & ITIS Catalogue of Life: 2011 Annual Checklist. Digital resource at www.catalogueoflife.org/annual-checklist/2011/. Species 2000: Reading, UK.

63-Encyclopedia of Life. Available from http://www.eol.org. Accessed 28 Jun 2011.

64-"What Is DNA Barcoding?." *Barcode of Life*. Consortium for the Barcode of Life , 2011. Web. 28 Jun 2011. <http://www.barcodeoflife.org/content/about/what-dna-barcoding>.

65-"Pioneer Species." *Forest of Leeds-Glossary*. Leeds, UK: Leeds City Council, 2006. Web. <http://www.leeds.gov.uk/fol/edu_gloss.html>.

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CHAPTER 7. TARGETS AND NIS BW DEVELOPMENT

1. INTRODUCTION

NIS (Non-Indigenous Species) used as BW (Biological Weapon) can be applied on a variety of targets. The targets would include food crops for humans or livestock feed. Also, livestock could be the intended targets as well as plants used for biofuel feedstocks; these biofuel plants include corn (*Zea mays*), sorghum (*Sorghum bicolor*), soybeans (*Glycine max*), sugar cane (*Saccharum*), or oil palm (*Elaeis guineensis*). Ecosystems could be targeted and the damage could be used in induce economic effects on ecotourism or psychological effects on target populations or as a means of ecological terrorism. Furthermore, damage to ecosystems would reduce biodiversity and deny potential resources from the biodiversity (e.g. new drugs, plant fibers, genetic strains, etc.). Also ecotourism, which in part is dependent on the biodiversity of a niche, would be seriously affected by an NIS BW attack on the biodiversity of a nation or ecosystem. Economy Watch (30) states that the ecotourism industry has experienced a yearly growth of 5% and at present the ecotourism market comprises nearly 6% of the GDP (Gross Domestic Product) across the globe. Finally, human populations or urban ecosystems could be targets for the purpose to spread disease or render a select area uninhabitable (i.e. Area Denial Weapons).

It is important to consider agricultural targets as part of the NIS BW attack strategy since an attack using BW on agricultural targets has been considered before (10, 11, 12, and 13).

Horn and Breeze (2) briefly describe how agriculture is one of the pre-eminent foundations for the United States' (US) wealth in the global marketplace as well as a key element for national security as part of US critical infrastructure. The US food and fiber system accounts for 13% of gross domestic product (GDP) and for 16.9% of total employment (2). Agricultural exports alone account for $140 billion and for 860,000 jobs. The United States has been known to have one of the most safe, secure, and reliable supply of food at a reasonable price that the world has ever known. Finally, the authors note that only about 2% of the population is involved in agriculture with the remaining population available to engage in business, commerce, and other wealth creating endeavors (2).

Yet, as Brown points out (3), much of the success in agricultural productivity and trade is dependent on freedom from disease. If disease enters the food production arena, both the consumer and the export markets are adversely affected. The spreading disease would affect the consumer with increasing food prices (especially as contaminated food stocks were recalled from shelves or culled from infected farms), while a simultaneous drop in export-market transactions would occur as nations refuse to import food stocks to prevent the spread of the disease to their own farms or morbidity or mortality of their own populace. Two brief examples warrant mention here.

Brown notes that the last major foreign animal disease outbreak in the US was avian influenza (1983-1984) in Pennsylvania and several neighboring states. After the expensive eradication of infected chickens and decontamination of chicken facilities was completed, the cost of the process was $63 million which was paid out by the US federal government; yet, during the six months period of the outbreak, the US consumer suffered poultry price increases to the total of $349 million (3). Yet, the impact on Great Britain due to bovine spongiform encephalopathy (BSE) was even more stunning. The emerging disease in cattle (prion based) required a mandated destruction of approximately 1.35 million cattle with all carcasses disposed of by incineration. This resulted in an estimated cost of over US$4.2 billion. Yet, as Brown notes, the cost in allowing prions into the food supply would have been devastatingly negative to the beef and dairy industries as a whole (3).

Parker (4) describes the "economic multiplier effect" of farm commodities as a measure of total economic activity of that commodity (e.g. eggs, grain, meat, milk). This multiplier effect starts at the farm gate value of the commodity and accrues value from transportation, marketing, and processing of the commodity. Parker states that the US Department of Commerce has concluded that the economic multiplier effect of exported farm commodities is 20 to 1 as compared to less than 2 to 1 for domestic crop sales and less than 3 to 1 for domestic livestock sales (4). It is this multiplier effect which helps to account for US agricultural product exports constituting 15 % of all global agricultural exports and (as noted above in US dollars export sales) making the farm component of the economy the largest positive contributor to the US trade balance (4).

The reasons for a BW attack on agriculture can be summarized by Chalk (1) who writes that three major outcomes would result from a bioterrorism attack on agriculture. First, economic disruption would occur creating at least three levels of costs. Initially these costs come from eradication and containment measures. For example, during the 1997 outbreak of Foot and

Mouth Disease (FMD) in Taiwan, the vaccination costs were $10 million, but the surveillance, cleaning, disinfection and related viral eradication costs were $4 billion. Second, the next costs are the indirect multiplier effects that would accumulate from both compensations paid to farmers for destruction of agricultural commodities as well as the revenue losses by direct and indirectly related industries (e.g. dairy processors, bakeries, abattoirs, etc.). Finally, international trade costs would occur due to protective embargoes imposed by major export partners. One example is the 1989 Chilean grape scare caused by anti-Pinochet extremists that laced fruit bound for the US with sodium cyanide. While only a small handful of grapes were contaminated, the resulting import suspensions (imposed by such nations as Canada, United States, Denmark, Germany, and Hong Kong) cost Chile over US$200 million in lost earnings (1).

Another possible outcome from a BW attack on agriculture would be the loss of political support and confidence in the government. Chalk (1) details how sociopolitical events, if not carefully controlled (including the media), would undermine the public's trust and cooperation in state and federal governance during the crisis. It is possible that euthanizing large numbers of animals to control the outbreak would result in such public distain that public protests could result to save infected animals or generate active resistance by farmers striving to protect infected herds from eradication (1). These public reactions could leave politicians with little strength to follow the necessary protocols to contain the epidemic lest they are voted out by an angry albeit poorly educated populace. Chalk provides an example of the 2001 FMD outbreak in Great Britain that triggered a massive public resistance to the livestock eradication and thereby resulted in a tremendous loss of public support for the Blair government and the Labor party in general (1).

The next outcome of a BW attack on agriculture is based on the motive of all terrorist attacks; to elicit fear and anxiety among the public. Chalk (1) mentions the effects could include socially disruptive migrations from rural to urban to escape the possibility of a zoonotic epidemic "jumping" species and becoming a human epidemic. This could be further complicated if the disease did in fact, jump the species barrier, or if it was genetically engineered to jump the barrier and infect humans as well as livestock. Chalk describes the example of the 1999 Nipah virus outbreak in Malaysia which not only destroyed the swine population of the Negri Sembilan province, but also killed 117 villagers. During the height of the outbreak, thousands of people deserted their homes and abandoned livestock while becoming refugees in shanty towns outside of Kuala Lampur (1). It must also be mentioned that a highly organized terrorist group could use social anarchists to help incite further social chaos by following the food attacks with riots over food shortages or price spikes. The scenario could be seen as step one: attack food stocks; step two: the attacks incite fear and terror in the populace; step three: orchestrate protests and riots against the government that the public does not trust; step four: cause violence during the riots to galvanize further mistrust of the government and cultivate further social chaos.

Chalk (1) finally discusses another outcome of a BW attack on agriculture: raising financial capital or blackmail. One possible route for a BW terrorist to raise financial capital would be to direct attacks which create and exploit fluctuations in the commodity futures markets. These attacks could be directed at crops or livestock or -even with the rise of biofuels- be directed against crops used for biofuels (e.g. corn or sorghum or sugarcane for ethanol production and soybeans or palm oils for biodiesel production). Either under direct support by other parties (e.g. organized crime, terrorists, foreign cartels) or acting independently, the BW terrorist would be able to take advantage of market reactions to the attack (as Chalk eloquently states "allowing the 'natural' economic laws of supply and demand to take effect") and harvest maximum dividends from the commodity futures sales (1).

Chalk (1) also observes that this form of BW terrorism could make it easier for state and federal government officials to negotiate with the terrorists (extortion and blackmail) to avoid the immediate and latent effects of the attacks. These forms of attacks would not garner the same public outcry over dead farm animals as they would have had over an anthrax or smallpox attack with numerous human causalities.

Finally, Hickson (5) discusses the use of BW against "soft targets" as a form of Fabian strategy of indirect warfare. In essence, Hickson describes the Fabian strategy (named after the Roman general Quintus Fabius Maximus, who defeated Hannibal by avoiding direct conflict) as a strategy of indirect actions used to weaken the resistance of an opposing force. If an aggressor wished to defeat an enemy, but avoid the "after effects" of prolonged direct warfare that would leave deep scars on the civilization or the subsequent peace; the aggressor must develop ways to weaken the enemy beyond their capacity to fight or beyond the capacity to sustain a prolonged fight (5). This strategy could include BW directed at agricultural targets with the resultant effects of reduced export trade of agricultural commodities, food shortages, reduced employment for workers in agricultural and food related industries, reduced biofuels productivity (if the targets include biofuels crops), and due to the multiplier effects, overall decreased economic vigor of the nation. This could result in a subsequent cascade of socio-economic effects, including as discussed above, distrust and resistance to state or federal government authority; greater social dissent exemplified by public protests over food or fuel shortages and spiking food prices; riots over unemployment or food shortages. These final actions could indicate to an aggressor that the enemy is now weakened sufficiently so that a quick invasion and defeat is possible.

2. FACTORS TO CONSIDER IN THE ATTACK AND SELECTION OF TARGETS

A. PROCESS OF ATTACK (SEE FLOW CHART 1: PROCESS OF NIS BW ATTACK)

The process of the attack is key in the developing an NIS as a Biological Weapon agent. Although the following two example processes are theoretical, the approaches could be used or modified depending on if the user was a nation state, non-state actor (e.g. terrorist group), criminal organization, or even a "long wolf" (individual) terrorist.

Preparation:

The target must be determined (e.g. niche, ecosystem, humans, urban ecosystem, agricultural field, livestock herd, or biofuel or fiber product). Furthermore, the mission objective (what is to be gained or achieved by this attach) needs to be carefully considered. In part, would the attack's purpose yield human or livestock fatalities or morbidities, destruction of agricultural crops, damage to the ecosystem or reduction of biodiversity (including loss of "ecotourism"), loss of market share for a crop, food stuff or biofuel feedstock shortage, or merely used to elicit fear in a local population or destabilize a government, economy, or international trade of specific goods.

As indicated, the potential ecological, economic and public health impact must be assessed before this process goes to the next step. This would include the economic multiplier effect if farm commodities were the target (4).

Furthermore, it must be clarified that once this NIS BW attack does occur, the method of surprise as well as the public and government shock having been achieved; but afterwards it will be lost in subsequent attacks as society will respond to the first attack with counterstrategies to prevent or manage subsequent attacks.

Step One:

This step requires collection of biological and ecological data of the target. It would include niche information. For example, is the niche urban, forest, pasture, agricultural fields, wetlands, etc.? What are the climatic variables to be considered (annual rainfall, yearly sunlight, etc.)? This data (biogeographical) is critical for successful GARP analysis or other ecological niche modeling. Also, target vulnerabilities would be considered. These include is the target disrupted by human activity (e.g. war zone, monoculture, construction, pollution, strip mining, deforestation, roadway construction, erosion) or has the target been subjected to wildfires, climatic changes, as well as reduced genetic diversity due to agriculture, tree farming, or limited reforestation efforts. Finally, other issues to consider would include knowledge of niche monitoring by scientific or government agencies as the monitoring efforts might detect NIS BW attacks or signal the need for counterstrategies such as eradication efforts. Also, another factor for consideration is whether the target area has large open field sites, such as livestock ranges, large monoculture fields (e.g. wheat, or other grains) or large industrial poultry coops.

Step Two:

This step would review the NIS candidate organisms. Factors to consider for the candidates would include previous NIS history (see discussion in Chapter 6); the ease of and time factor for cultivation of NIS propagules; would propagule dispersal require single or multiple discharges on the target sites; what format would the propagules be dispersed as (e.g. bacterial cells, endospores, seeds, spores, vector borne, or adult organisms, etc.); means by which the NIS can reproduce (asexually or sexually); presence of generalist or pioneer traits in the NIS; absences of enemies (3P's) in the target niche; if the related species to the candidate organism (by family or by genus) exhibited prior invasiveness traits; and ease of transport, storage, and delivery of the NIS propagules. Furthermore, it must be considered that if the NIS candidate requires a vector for successful delivery and colonization, then consideration of the ease of vector culture and introduction (e.g. infection) and stability of the NIS candidate in the vector must be considered. Finally, the candidate consideration must include time lag until colonization has occurred, rate of spread of NIS in the target niche and beyond; time lag of NIS BW attack until discovery of the NIS; and NIS potential damage to biotic and abiotic components of the target niche (e.g. morbidity, biodiversity, soil chemistry alteration, phytopathology, economic disruption, etc.).

Another factor to consider is the time of dispersal of the NIS as a BW attack. Sequeira (6) discusses that one of the variables in a successful NIS introduction is the precise timing of the NIS release to occur at a time for maximum colonization (6). For example, Baskin (7, pg 205) describes the seasonality of the Papaya Fruit Fly (*Toxotrypana curvicauda Gerstaecker*) invasion in Australia as associated with the wet season where the fly has the best chances of colonization. It is during this wet season in Australia that active growth of annuals and fruiting of trees-the fruit fly food sources- occurs (7, pg 205).

After NIS invasion has occurred (i.e. NIS BW attack), one other factor to consider is whether government, academic or environmental organizations or agencies have had previous experience with an NIS invasion of this candidate (hence, possible early detection and activation of eradication efforts for this organism).

Step Three:

The analysis of the data from the previous steps would help in the decision of whether the NIS candidate would meet the criteria of the mission objective. A final review would include a GARP (or other type of ENM) analysis for invasion success of the NIS BW attack. As noted in Chapter 6, if the attack is short lived and even if it does not yield colonization, the psychological effects could yield long term effects of market instability or panic of the local populace. Kadlec (8) uses one example of a 1993 insect attack on Pakistani cotton crops which caused long term economic ripples on subsequent Pakistani cotton exports. Since farmers reduced cotton planting in subsequent years to reduce risk of crop failure and shifted to less preferable yet more reliable crops of rice, wheat, and sugarcane, the effect was a significant decline in exports of a key cash crop-cotton (8).

If GARP NIS analysis indicates colonization success in the theoretical niche sites, then the analysis supports the target and mission objectives. Once the analysis steps are complete and the data supports the target niche of the NIS BW attack and the mission objectives, then the NIS production step begins.

Replication of NIS and/or Vector:

The steps for replication of the NIS and/or vector will depend on the number of propagules required as well as the type of NIS required. A naked NIS BW agent (i.e. does not require a vector) can be for example, seeds, spores, viruses, or even adult organisms. NIS organisms that require a vector, may require the culturing of both the NIS and vector (e.g. mosquitoes, ticks, flies, plant seedlings, etc.) as well as time to co-culture together (e.g. infection) the NIS with the vector carrier. This process may be more costly, labor intensive, and difficulties may arise if the NIS organism and vector are not easily capable of incorporation or if the NIS/vector combination is not stable for extended periods of time prior to target dispersal.

As noted previously (see Chapter 3), BW using insects and other organisms requires knowledge of the mass cultivation of the organisms. For example, Lockwood (9) notes that techniques to mass cultivate and use the Colorado potato beetle (*Leptinotarsa decemlineata*) as BW were developed by Nazi Germany. Yet, the French prior to World War II had also developed mass cultivation of the same beetle and later the United States and Soviet Union explored and developed mass cultivation techniques for a variety of insects (e.g. mosquitoes, fleas, flies, etc.) and pathogenic organisms (8, 9, 10, 11, 12, and 13). The cultivation information is relatively easy to obtain for many organisms as the mass production techniques would have a dual-use in the research and development of insecticides and other insect pest treatments. Even the culture of the Colorado potato beetle (*Leptinotarsa decemlineata*) has been perfected using artificial feed as demonstrated by the work of Martin and Gelman (14, 15).

Finally, if multiple releases of propagules are necessary, the timing of the releases would influence the timing of the culturing of the NIS and/or the vector.

Method of Dissemination of NIS:

The methods for dissemination are varied. These methods will vary depending on whether the NIS BW user (aggressor) is a nation state, rouge nation, non-state actor (e.g. terrorist), criminal organization, or a lone individual (aka lone wolf). The variation in this factor is dependent on the resources available to the aggressor (e.g. funds, manpower, technology, smuggling resources, etc.).

The methods of dissemination are also influenced by the actual NIS BW agent (e.g. seeds, spores, viruses, plants, insects, etc.) and whether or not the NIS organism requires a vector carrier. As stated previously, simple smuggling into a country by covertly evading border and/or Biosecurity agents and protocols would be a common strategy (7). Smuggling could also occur under the guise of imported goods, imported herbal remedies (e.g. plant seeds or dried plant material), in traveler's packages or suitcases, or even commercial container ship ballast water (7, 16, 17, and 18). Any of these delivery routes might be able to bypass Biosecurity protocols with the proper planning. Baskin (7, pg 116-117) even described how NIS wildflower seeds were mailed to Hawaii and barley seeds (with potential NIS fungal pathogens) were mailed from New Zealand to overseas nations including Australia. In a sense not unlike the 2001 US bioterrorist attack with Anthrax laden letters, the postal services could be used to deliver NIS BW propagules to unknowing or knowing recipients (33).

Another route for consideration would be the use of migratory species (e.g. birds, butterflies, fish, etc.). The factors to consider in using this strategy would include the size and type of NIS BW organism to "hitch a ride" on the migratory species; whether the migratory species would be affected by the NIS BW presence (e.g. morbidity, mortality, etc.). Also, would the migrating species pass into the desired target objective niche and for a long enough period for the NIS BW to be deposited effectively in the target zone? Mack (16) briefly mentions that migrating species have played a role in the distribution of plant species across the globe. Essl et al (32) briefly mentions that the dispersal capacity of NIS birds and insects can enhance the exploration of habitats, the expansion of invasion sites, and accelerate naturalization in new habitats.

For example, Schmann (19) describes how the common barberry plant (*Berberis vulgaris*), an NIS originally from Europe, was an alternative host to the wheat stem rust fungus (*Puccinia graminis f. st. tritici*) and hence a threat to the American wheat crop. To control the spread of wheat rust, a barberry eradication plan was implemented across the United States in 1918 (19). Although mostly successful, the eradication is not complete as various birds can consume the common barberry fruits and disperse the seeds as they migrate or travel locally (20, 21). This is a form of endozoochory (i.e. seed dispersal via ingestion by animals).

Finally, dispersal could occur by more technical and precise methods. As previously discussed in Chapter 3, a Biocruise missile (22, 23) as a dispersal vehicle guided by Global

Positioning Satellite (GPS) navigation would be a superior method to accurately and rapidly deliver the required amount of NIS BW agent on the target niche. This would depend on the size of the NIS BW agent and the number of propagules of the NIS BW required, but if the agent is small enough to be carried as a biocruise payload and properly dispersed from the missile, the NIS could be very precisely delivered to the target and even multiple dispersals on the same site, or large propagule numbers in a single discharge, or dispersals over multiple sites per missile could be possible.

It has not been left unnoticed that the technology of delivery systems has become more sophisticated in the past decade. At present, as new technologies to deliver payloads have been developed; some offer stealth technology as part of the delivery system. Two technologies merit mention at this time.

An Israeli company, Nanoflight, (24, 25) reports the development of a nanotechnology based paint coating capable of rendering drones, missiles or aircraft difficult or impossible to indentify or track by radar. The coating absorbs the microwave radiation (radar) and transduces the energy into heat energy which dissipates to the surrounding space. The result is that any flying object cannot be adequately identified or properly tracked by radar. Any dispersal device coated with this nanotech coating during an NIS BW attack could disperse the agent without effective detection.

The second technology is a cruise missile system from the Russian company, Contsern-Morinformsistema-Agat, called Club-K (26). Stored in a standard 40 foot shipping container, the cruise missile system contains four missiles, missile launch rail system, and launch control module. The missile container system can be easily transported and launched from flat bed truck, rail, or ship (27). This system, costing about $10-20 million, can launch cruise missiles with a range of 400 kilometers and one anti-ship version carries a second stage which detaches after launch and accelerates to supersonic (Mach 3) speed (26, 27, and 28). The Russian company promises not to sell the system to terrorists (28), but other weapons specialists and military advisors are doubtful (28, 29). If this system was adopted for NIS BW, an attack using NIS BW could be done from offshore on a cargo ship, or transported via rail or container truck and launched with no warning. With the container format of the system as cover, the system would be concealed from counter-terror or defense agencies.

Testing:

The testing of a NIS BW agent might not be necessary. This would depend on the available data collected during the NIS BW decision process; whether the aggressor has the means and resources to perform a test (including suitable site similar to the target site); and whether the test is necessary using the actual NIS BW organisms or would a “dry run” be only necessary to test out the delivery protocols (e.g. smuggling steps) to test border or Biosecurity protocols. It may also be necessary to test the delivery vehicle (if one is used) carrying capacity and dispersal unit (e.g. sprayer or payload drop method).

It must be recognized that the risk of any testing with an actual NIS BW includes the possible discovery by other organizations or nations of an impending NIS BW attack by the aggressor. In essence, the secrecy of an NIS BW attack would be compromised.

Distribution of NIS (ACTUAL BW ATTACK):

The actual NIS BW attack could take several stages depending on the aggressor’s resources (e.g. comparative resources of a nation-state versus terrorist group versus lone individual, etc.) and the manpower required for successful delivery; the number of propagules required for successful NIS BW invasion and colonization; the size and lability of the NIS and/or vector required; and time lag for invasion, colonization, and discovery of the NIS BW attack.

Analysis of Results:

Depending on the time lag of the NIS organism, the analysis of the actual NIS BW results may range from weeks to months to years. The variables of analysis would include the target selected (e.g. human, livestock, ecosystem, field crops, etc.), and the mission objective (e.g. public health effects, economic effects, biodiversity damage, etc.). It must be also noted that due to the time lag from the actual NIS BW attack, the aggressor may never need to nor want to admit culpability of actions depending on the means of delivery and the mission objectives. The aggressor may simply wait until the effects of the NIS BW invasion or colonization cause the outcomes intended (e.g. economic market shifts, social panic, disease outbreaks, niche collapse, etc.).

B. PROCESS OF ATTACK-INVASION MELTDOWN

(SEE FLOW CHART 2- MULTIPLE NIS SPECIES IN BW ATTACK TO ACHIEVE INVASION MELTDOWN)

Although by comparing Flow Chart 1 with Flow chart 2, the steps are nearly identical, it merits discussion on the key step differences and unique steps that make an invasion meltdown different from a simple NIS BW attack. The key difference is that in an invasion meltdown (as discussed in Chapters 4 & 6), multiple NIS species contribute synergistically to accelerate the impact on the naïve ecosystem (31).

The following steps are similar and differences are noted where necessary:
-Target Determination & Mission Objectives
-Step 1- Biological and Ecological Data of the Target

-Step 2- Selections of organisms –NOTE: plural as it is here that various NIS candidates are considered to be used on the same target. Also, for each NIS species, the factor of multiple propagules will need review.

-Step 3-Questions to be considered include: Will the multiple NIS candidates meet the mission objective? Further, will their effect due to invasion meltdown speed up the rate of colonization and accelerate the rate of impact of the mission objective? A GARP analysis of each NIS species would be required with the results favoring a high probability of overlap of all NIS candidates on the target niche.

-Step 4- This is a key step. Analysis must be done to determine (historically from previous data or potentially) if the NIS organisms would synergistically enhance or interfere with each other species invasion and subsequent colonization. If the NIS species candidates and potential for invasion meet the target determination and the mission objectives, then the NIS organisms and (if necessary) the vectors are to be replicated.

-Replication of NIS and/or vector carriers- similar (see comments in next stage)

-Determination of method of dissemination of NIS- In the determination of replication factors and in the methods of dissemination of NIS species, several factors need to be considered.

-Temporal factors-the timing (including seasonality) in both replication and dispersal of the NIS organisms.

-Can multiple NIS species in whatever form (seeds, spores, etc.) be distributed together or separately over a time period as well as stored in separate containers or mixed together in the dispersal vehicle?

-Testing stage- comments are effectively the same as the single NIS BW approach (see above).
-Distribution of NIS (Actual BW Attack) - Distribution of the NIS may require multiple dispersals of the NIS species as well as increased number of propagules per species to ensure successful invasion for subsequent interaction during invasion and colonization.

-Invasion Meltdown- This step may be delayed by a time lag, but that will depend on the NIS species selected. The time lag may be as long as single species NIS BW attack, or much shorter in time as the synergistic effects of the NIS BW species alter the niche at a greater rate of speed then if a single NIS species was present. The multiple species attack could also multiple mission objectives (e.g. human disease and livestock morbidity, food crop and biofuel crop destruction, damage to forests and agricultural fields, etc.).

-Analysis of Results- The comments are similar, yet with invasion meltdown, it is suspected that the synergistic interactions of multiple NIS species would result in more shortened time scale of observable results. The results would have serious effects on economic markets, ecological systems, and public health in general.

C. VULNERABILITIES OF NATIONS AND NICHES

Vulnerabilities of any nation or niche can contribute to the threat of an NIS BW attack. The vulnerabilities vary from nation to nation across the globe. As Pravecek and Davis (40) paraphrase a threat determination formula devised by Lt. Col. Don Noah, USAF in chapter 3; the formula for a threat consists of an adversary's *intent* to use BW; an adversary's *capability* to use BW; our own *vulnerability* to BW, equals the threat. If this vulnerability is due to poor Biosecurity, poor border control, poor environmental monitoring, lack of scientific training on

NIS, or lack of funding to control invasions as comprehended by an aggressor (as well as the aggressor's intent and capability for NIS BW are equally high), then the threat level for a NIS BW becomes very high and very real. Whitby (10) describes the vulnerability factor from the observation that developing countries are increasingly dependent on the production of a single staple food crop (in part, due to lack of resources for agricultural extension stations and other research tools); whereas, the more advanced developed nations have the resources for agricultural research and innovation and can afford producing a number of staple food crops. Whitby notes that it is these resource limitations and single staple food crop production dependency that makes less developed nations more vulnerable to agriculture BW (including NIS BW) (40).

Many niches exist in nations with limited resources (e.g. funds, scientific training, border security, Biosecurity policies, etc.) to maintain Biosecurity. Even those nations with legislation, trade policies, border agents, and Biosecurity practices like the United States, Australia, or South Africa (34, 35, 36, and 37), still have NIS organisms slip in through via accidental or deliberate means. Furthermore, one study by the National Research council noted that recent NIS introductions in the US are not merely from Europe or China, but appear to have been influenced by Caribbean, Asian, and other immigrant groups (38). Furthermore, Oppel et al (39) studied the challenges to eradication of NIS mammals in islands occupied by humans and domestic animals. Oppel and team found that humans indirectly supported NIS organisms by the presence of trash, garbage disposal areas and livestock feeding areas (which supplied food and shelter to the NIS mammals) as well failed to monitor transports to the island which could reintroduce NIS mammals (i.e. multiple propagules). But what was more surprising was the substantial opposition to eradication efforts by those who opposed animal cruelty, were concerned about animal welfare, filed lawsuits to alleging animal cruelty, or objected to use of poison baits out of human health concerns (39). If these policies existed at the target site for an NIS BW attack, it would obstruct organized eradication efforts as well as undermine efforts directed to preventing the spread of the NIS species and preventing niche damage.

Other vulnerabilities included niches with fragile ecosystems or limited biodiversity (e.g. simple food webs with low number of nodes) which under the proper NIS BW attack would suffer a greater impact by the NIS. Islands with unique endemic species have limited biodiversity and are vulnerable to NIS invasions. Baskin describes the invasions over four centuries by ships and travelers to Hawaii and the impact to native species and niche destruction that has occurred (7, pg 73-74).

Another vulnerability for a nation is the poor communication of scientific or government agencies, a type of organizational dysfunction. Many of the necessary NIS protocols and details may not be effectively managed or properly controlled due to mismanagement or poor development of government regulations to prevent NIS invasion or manage NIS invasions after the fact. Goku (41) described how the 2004 Japanese "Invasive Alien Species Act" was enacted to control NIS invasions, but a loophole existed that does not address alien micro-organisms. Using the NIS invasion of the amphibian chytridiomycosis (*Batrachochytrium dendrobatidis*) as an example, Goku explains that despite Japan's dependence on imports (including more than 500 million live animals each year), the act lead to confusion among scientists and the Japanese Government as the act never anticipated alien micro-organisms as a threat (41).

If there is a lack of resources in the public health sector or in environmental monitoring, this would be a vulnerability to preventing an NIS BW. If the public health monitoring (or veterinarian monitoring services) cannot detect an upswing in cases of a disease distributed by the NIS BW or if the environmental services or non-governmental environmental or academic researchers do not have the resources to detect an NIS BW attack, then the effects of the attack may have time to achieve colonization and further niche damage in many forms-economic, ecological, and public health.

D. NICHE RISK FACTORS

As discussed in Chapter 4, a risk assessment formula and the means to estimate out the NIS risk threat of an organism have been developed (42, 43). Furthermore, the risk of a niche to a particular NIS has been further understood using Environmental Niche Modeling (ENM) as described in chapter 6. Yet, it is worth briefly mentioning that individual niches can have unique properties that make the niche more susceptible to NIS invasion and colonization.

One consideration is limited genetic diversity in the niche. If one considers an agricultural field an example of a niche, the niche demonstrates monoculture of the crop. Monoculture is the farming practice where only one crop is raised in a field (e.g. wheat, corn, tomatoes, barley, etc.) and many times the crop grown is a hybrid strain that exhibits genetic uniformity (i.e. very narrow genetic diversity) (19). As a result, the monoculture crop becomes a large scale susceptible host to the pathogen infection (in this case NIS invasion) and spread of the pathogen (NIS propagules) within the monoculture field (4, 10, 12, and 19). NIS invasions favor low genetic diversity (7, 38). If the pathogen (or NIS) can spread beyond that field by airborne particles for example (such as fungal spores), then the pathogen can successfully spread to other fields or across the country or even across the continent. Dudley and Woodford (47) raise the issue of vulnerability due to limited genetic diversity in livestock and how selective market pressures and in-breeding have resulted in Europe having a very limited genetic diversity of livestock (e.g. cattle, hogs, sheep, etc.) . This limited genetic diversity makes the livestock prime targets for NIS BW attacks. The authors also note the concern that BW attacks could not merely damage biodiversity, but cause extinction of endangered wildlife species (47). Kolar and Lodge (48) assert that NIS are recognized as one of the top global threats to native biodiversity and ecosystem function.

Another niche factor is the presence of disturbed regimes. Disturbance regimes are sites of disruption of the biotic and abiotic conditions of the niche. Common disruptions of ecosystems include roads, natural disasters (like fire, droughts, or floods), and polluted ecosystems. Hansen and Clevenger (44) observed that in transportation corridors which create disturbance regimes in plant communities along the corridor edges, the probability of invasive species establishing and spreading is greatly increased as compared to control sites or to habitats a significant distance from the corridor site. Mack and D'Antonio (45) reviewed various studies of human activities and the intensity of ecological disturbances. One interesting additional observation the authors reported was that human activities could disturb ecosystems by the introduction of invasive species (45). The studies indicate that NIS modification can restructure the ecosystems by modifying disturbance regimes or adding new disturbances to the ecosystems (45). Kimberly With (46) devised a means to estimate the thresholds of NIS colonization and spread in fragmented landscapes (concept similar to disturbed regimes). By comprehending the relative effects of landscape structure on the processes that contribute to NIS spread, With was able to determine that colonization success is highest when over 20% of the landscape is disturbed (especially if the disturbances are large or clumped together) (46). This is due in part to the probability that NIS propagules will likely find favorable sites in the disturbed patches. Also, the invasibility of communities (success of NIS invasion) will be greatest in landscapes with concentrated areas of disturbance, especially if the disturbance has rendered the site below the critical threshold of biodiversity. In that case, it is possible that a single NIS invasion can trigger a cascade of extinctions among native species in that site (46).

Although more research would be needed and welcomed in these topics, the present data offers interesting insights into NIS BW applications and niche vulnerabilities. It is conceivable that a bioterrorist would first damage a niche by initiating a wildfire. After the fire damage, and if the critical threshold of biodiversity is reduced to vulnerability, then a follow up NIS BW attack could conceivably wipe out the remaining autochthonous species in the niche.

3. FOUR EXAMPLES:

It is worth examining several examples of possible NIS BW attacks based on data from various sources. Although more research-especially GARP analysis or other forms of ENM-is necessary, it would be interesting to explore these scenarios as possible models of future NIS BW attacks. Where possible, any data using ENM or previous NIS history will be mentioned in the construction of these NIS BW examples.

A. NIPAH VIRUS AND NIS PIGS

The Nipah virus is a paramyxovirus, first recognized in Malaysia in both humans and pigs in 1998-1999 and later in Bangladesh in recurrent outbreaks from 2001-2007. (49, 50). Although the reservoir host is fruit bats of the genus *Pteropus*, the virus can infect pigs, both domestic and feral, as well as humans (49, 50). Evidence exists that not only pig to human transmission occurs, but human to human (49, 50) as well as pig to other wildlife or domestic animals (E.G. cats, dogs, etc.) (50, 51). Weingartl et al (52) found that experimental infection of pigs and cats can occur orally, oronasally, ocularly, or subcutaneously (hence aerosol transmission by coughing pigs was believed to be major means of transmission to farm personnel). The researchers also found the Nipah virus is up to 100% infectious in pigs, yet the mortality ranges from 1-5% and both infected bats and pigs can appear asymptomatic (52). The Nipah virus can persist over a long period in the patient before causing fatal disease (52). Nipah will infect both the respiratory and neurological systems (53). One study in Bangladesh found that of the 122 Nipah cases identified, eight-seven patients (71%) died; which suggested Nipah is an agent with high mortality (50). The Centers for Disease Control (CDC) has classified the

Nipah virus as a Category C bioterrorism agent due to its availability, ease of production and dissemination, and potential for high morbidity and mortality rates and major health impact (54).

The wild pig (*Sus Scrofa*) was first introduced to the United States from Europe in the 1500's, originally as escaped domesticated pigs (55). Later, German wild pigs were released into New Hampshire in 1893 and in the early 1900's, Russian wild boars were released for gaming purposes in California and North Carolina (56). It is a known NIS in US ecosystems. These wild pigs and their hybrid offspring have become a problematic NIS now spanning 39 states in the US (55), including New Hampshire, California, Texas, Hawaii, and Florida (56, 57). The wild pigs are dietary generalists that can consume wild animals (e.g. deer, quail, snakes, etc.), young livestock, can damage farm crops as well as ecosystems, and can scavenge for carrion if necessary (55, 56, and 59). Only two generations are necessary for escaped domestic pigs to revert to feral pigs (55). Wild feral pigs also can carry a variety of diseases including Pseudorabies, Swine Brucellosis, *Toxoplasma gondii*, and *Trichinella* (58). These diseases can be transmitted to other domesticate livestock, humans, and other wildlife (55, 56, and 57).

If an NIS BW attack would to use the Nipah virus as the NIS on a present NIS species as the feral pigs, the disease could be presented by injection to trapped animals or by aerosol of a sounder (i.e. large group of pigs). The disease would spread into the ecosystem, human farming population, as well as the domesticated pig population. Over time, if the feral pig population survives the initial introduction of the virus, the asymptomatic pigs could spread the virus throughout the surrounding states and the virus would become endemic in the ecosystem; as well as become a public health risk to abattoir workers, farm workers, and hunters; and negatively affect international economic trade of pork related products . It is also possible the presence of the virus with a human to human aerosol transmission and moderately high mortality rate would cause social panic in an outbreak.

B. STRIGA AND CORN CROPS

Striga species (commonly known as Witchweed) is a plant parasite with a crude root system that invades another plants' root system for nutrients, eventually stunting growth and killing the host plant (60, 61). Striga species are native to Africa, although some species are native to the Indian subcontinent, and Australia (60). The seeds of *Striga* are tiny, about 0.3 millimeters long and 0.15 millimeters wide, with a single plant producing 40,000 to 90,000 seeds per plant (depending on the species of *Striga*), and the seeds can remain viable in the soil for up to 20 years (62). The host crops of *Striga* strains include major cereal, food stuff, and biofuel crops: corn (*Striga asiatica, Striga hermonthica*), Rice (*Striga hermonthica, Striga asiatica*), Sugarcane (*Striga curiflora, Striga hermonthica, Striga asiatica*), sweet potato, (*Striga gesnerioides*), and Sorghum (*Striga aspera*, *Striga hermonthica, Striga asiatica*) (60, 62). GARP analysis of the NIS global invasive potential of Striga, including for the United States and Mexico has been done (63, 64). Striga invasion into the US Corn Belt would threaten the corn crop valued at $20 billion annually (64). Except for the extreme northern most US, *Striga asiatica*-using GARP analysis- would become a destructive pest to the US corn belt-not to mention the sorghum and rice production in the same states. Previous experience with a small invasion of *Striga asiatica* in an area of eastern North Carolina has proven very difficult to eradicate, especially for the reasons previously mentioned regarding seed viability and seed proliferation (65). Using artificial stimulants (strigol) and selective herbicides, the witchweed invasion has been stopped in eastern North Carolina fields (65).

BUT, if a NIS BW attack of the *Striga* species were used in a hand dispersed or even aerial dispersal methods (e.g. Biocruise using GPS methods to pin point target large corn fields), the impact on US corn production due to large outbreaks of *Striga* would crush the corn market in the US and seriously impact corn-based biofuel production. The United States Dept. of Agriculture (USDA) projects that from the 2011/2012 corn harvest, 50 million bushels of corn will be converted to 132 million gallons of ethanol (for a corn to ethanol biofuel conversion rate of 2.7 gallons per bushel) which equals about 3 million barrels of ethanol biofuel or roughly equivalent to 10% of the monthly US oil imports from Saudi Arabia (75).

Furthermore, the *Striga* attack would create a crisis in the international trade of corn and the US balance of trade which is heavily dependent on agricultural exports (including corn). The corn market is so critical to US economic security that Kadlec (8) in his scenarios of BW attacks used to create economic warfare focused on corn markets in two of his scenarios. It is important to note that Kadlec (8) used corn blights as the weapon of choice in the scenarios, whereas with *Striga;* due to the high propagule numbers (i.e. seed production) and longevity of seed viability in the soils; the *Striga* attack might destroy the prolific US corn harvests for decades as well as render the fields useless (i.e. similar to an “area denial” weapon).

C. TROPICAL BONT TICK AND HEARTWATER

The Tropical Bont Tick (*Amblyomma variegatum*) is an NIS in the Caribbean islands and was originally imported on cattle from Senegal, West Africa onto the island of Guadeloupe in 1830 (66, 68). Tropical Bont Tick (TBT) has spread to 19 islands in the Caribbean and is a potential threat to the United States wildlife and domestic livestock (67, 68). TBT is a 3-host tick with a wide host range that can include to cattle, sheep, and goats as well as various wildlife including: jackals (*Canidae*), hares (*Leporidae*), Zebras (*Equidae*), Antelope (*Bovidae*), storks (*Ciconiidae*), mongooses (*Viverridae*), African green monkey (*Cercopithecus sabaeus*), black rat (*Rattus rattus*), cattle egrets (*Bubulcus ibis*), Norway rat (*Rattus norvegius*), house mouse (*Mus musculus*), white-tailed deer (*Odocoileus virginianus*), and African buffalo (*Syncerus caffer*) (68). Cattle egrets have been found to disseminate the ticks among the Caribbean islands as well as ground dwelling birds (68). During dispersal and migration of cattle egrets, larvae and nymphs of TBT have been found to survive and the recent expansion of TBT in the Caribbean islands has followed the migration of the egrets (68). Furthermore, one recent report of a cattle egret with TBT was found to have migrated to the Florida Keys from the island of Guadeloupe (69).

TBT is a major vector of the rickettsial disease, Heartwater (*Ehrlichia ruminantium*- formerly *Cowdria ruminantium*) (71). Heartwater is an important cause of death for cattle, sheep and goats in Africa (70). Heartwater has been found to infect rodents, reptiles, birds, lagomorphs, and certain carnivores (71). Heartwater rickettsial organisms infect endothelial and white blood cells (71). Postmortem of cattle demonstrate edema in the CNS and in the pulmonary region; hence the name "Heart water"(70). Furthermore, Burridge et al (69) has demonstrated that two imported reptilian tick species (African Tortoise Tick, *Amblyomma marmoreum* and Central African tortoise tick, *Amblyomma sparsum*) that have been established in Florida (hence NIS), are experimental vectors for Heartwater. Ten African Amblyomma species, including TBT, are known to transmit Heartwater (71). Furthermore, Uilenberg (71) reports that endemic stability of Heartwater can occur in cattle exposed to large numbers of infected TBT and thus, ruminants that recover from initial infection have been discovered to remain long-term carriers of *E. ruminantium* (71). One further issue confounding risk analysis of Heartwater and TBT is that recent studies in Zimbabwe have reported that some cattle carriers of Heartwater are seronegative; hence seronegative results from current Heartwater tests do not indicate absence of Heartwater infection (69). Also, vertebrates other than mammals can be carriers of Heartwater, including tortoises and indirectly egrets and other migratory bird laden with *E. ruminantium* infected TBT (71, 72). *E. ruminantium* can persist in ticks for up to 15 months (73). Burridge (72) in 1997, warned of the threat looming to US livestock and deer population from Heartwater. Finally, Allstopp et al (74) reports initial molecular data of three deaths of healthy children in South Africa suspected to be due to *E. ruminantium.*

If TBT infected with *E. ruminantium* was introduced into the US, it could rapidly spread into both wildlife and domestic cattle ranges with severe effects on cattle and sheep farming as well as severely impacting wildlife including deer, birds, reptiles, as well as in various niches, rabbits and rodents. The Heartwater disease would eventually become endemic in various wildlife niches in the Southern and mid-western US niches and could possibly infect humans. The primary impacts in this NIS BW attack would be the cattle and sheep markets, the beef industry, as well as the ecological damage to various wildlife species. This dispersal of TBT laden with Heartwater could be a simple smuggling task of infected ticks and other tick laden birds or cattle into the US as well as using migratory birds laden with TBT (infected with Heartwater) to cross into the Southern US. Advanced technologies (e.g. aerial dispersal, biocruise, etc.) to disperse large qualities of TBT infected with Heartwater would require more advanced resources, but could allow for a highly accurate dispersal of TBT into cattle ranges in the Southern US, resulting in a more rapid NIS BW invasion, colonization, and economic impact.

D. BARBERRY AND WHEAT STEM RUST

The Common Barberry (aka European Barberry) plant is an NIS in the US originating from Central and Southern Europe. The Common Barberry (*Berberis vulgaris*) is present in all New England states, most northern states, and many southern states including South Carolina, Missouri, and New Mexico (20). Despite eradication efforts, various birds can consume the common barberry fruits and disperse the seeds as they migrate or travel locally (20, 21). The barberry can proliferate in a variety of habitats including: pastures, wetlands, roadsides, vacant lots, gardens, floodplain forests, open-canopied forests, early successional forests, and coastal grasslands (21). The Barberry is the alternative host for the Wheat Stem Rust fungi. As Schumann (19) noted, US efforts to eradicate the barberry were fueled by the effort to reduce the

Wheat Stem Rust in the early 20th Century. Wheat Stem Rust (aka Black Stem Rust of Wheat) reduces the yield of wheat-both quantity and quality- as the uredial eruptions on the stem cause the stems to fall over making any harvest impossible (19).

The life cycle of the Wheat Stem Rust (*Puccinia graminis f. st. tritici*) is that basiodiospores infect barberry plants and create aeciospores which infect wheat plants and create uredial pustules on wheat stems (19). These uredial pustules produce uredospores that provide a "repeating stage" of infection for the wheat plants and results in wheat field epidemics (19). These uredospores can spread great distances via winds and transported northward by higher air layers (78). Later in the season, the dikaryotic mycelium in the wheat stems create thick walled teliospores that survive the harsh cold winters (19). The teliospores produce basiodiospores in the spring to start the cycle over again (19). If the barberry was absent (via eradication), the teliospores would produce basidiospores, which would not continue the life cycle and the previous season's uredospores would have perished during the harsh winters (19). Hence, the rust epidemic could die out. Klinkowski (78) notes that Wheat Stem Rust can overwinter on winter wheat in southern Texas. A paper by Madden and Van den Bosch (77) describes how rust diseases have lower economic impact since their overwintering potential is low without overwintering hosts. The presence of barberry enhances the long term economic impact of wheat stem rust as the alternative host provides between season survival and reduces the risk of extinction to zero (77).

It must be noted that wheat stem rust was previously developed as a BW agent. For example, Whitby (10) noted large scale production of Wheat Stem Rust uredospores by the US military in the 1950's. The techniques for the cultivation of various spore stages are publically available. For example, Pillai et al (76) describe laboratory methods to product wheat stem rust teliospores.

If an NIS BW attack was initiated (e.g. United States) using wheat stem rust in combination with common barberry to establish invasion and colonization (infection) beyond one season and create the conditions for the dispersal of uredospores via winds to expand the NIS BW attack across wheat fields at great distances. This process would first require cultivation of Barberry seeds for distribution (to enhance the wheat stem rust infectivity beyond the first season). The wheat stem rust teliospores would be the spore format that is hardy enough to withstand aerial dispersal and distribution during less favorable seasons (uredospores- would be favored if cultured and distributed during summer months). Distribution could occur by hand, but aerial dispersal is favored (e.g. Biocruise using GPS methods to pin point target large wheat fields) as it could be used in either wheat fields or niches (e.g. meadows, forests, etc.); furthermore, the uredospore or teliospore distribution should be within range to subsequently infect wheat fields. One other factor to explore is the temporal issue: can barberry seeds and rust teliospores be distributed together; or would barberry seeds require initial release to create a receptive NIS alternative host site that is receptive to the subsequent teliospore distribution.

The results of a successful NIS BW attack using a combination of common barberry and wheat stem rust would be the decline of wheat harvests, price rise in wheat based food products, and market effects on wheat commodities-especially due to fears of wheat harvests contaminated with wheat rust spores. This last situation could lead to global export ban of infected crop to prevent spread of the wheat stem rust. Furthermore, one strain of wheat stem rust, Ug99, is of great concern as present research indicates it is highly infectious and leads to severe epidemics (79). The United Nation's Food and Agricultural Organization (FAO), reports that the Ug99 strain of wheat stem rust has migrated from Africa into Iran (a major wheat producing nation) and furthermore threatens other major wheat producing nations in central Asia (80). With no established wheat strains that are resistant to Ug99, the introduction of this fungal strain as part of an NIS BW attack would wreck havoc on global wheat markets. Furthermore, as the disease spreads and the wheat harvests decline, the social effects would include fear of famine or actual panic due to rising food prices.

4. SUMMARY

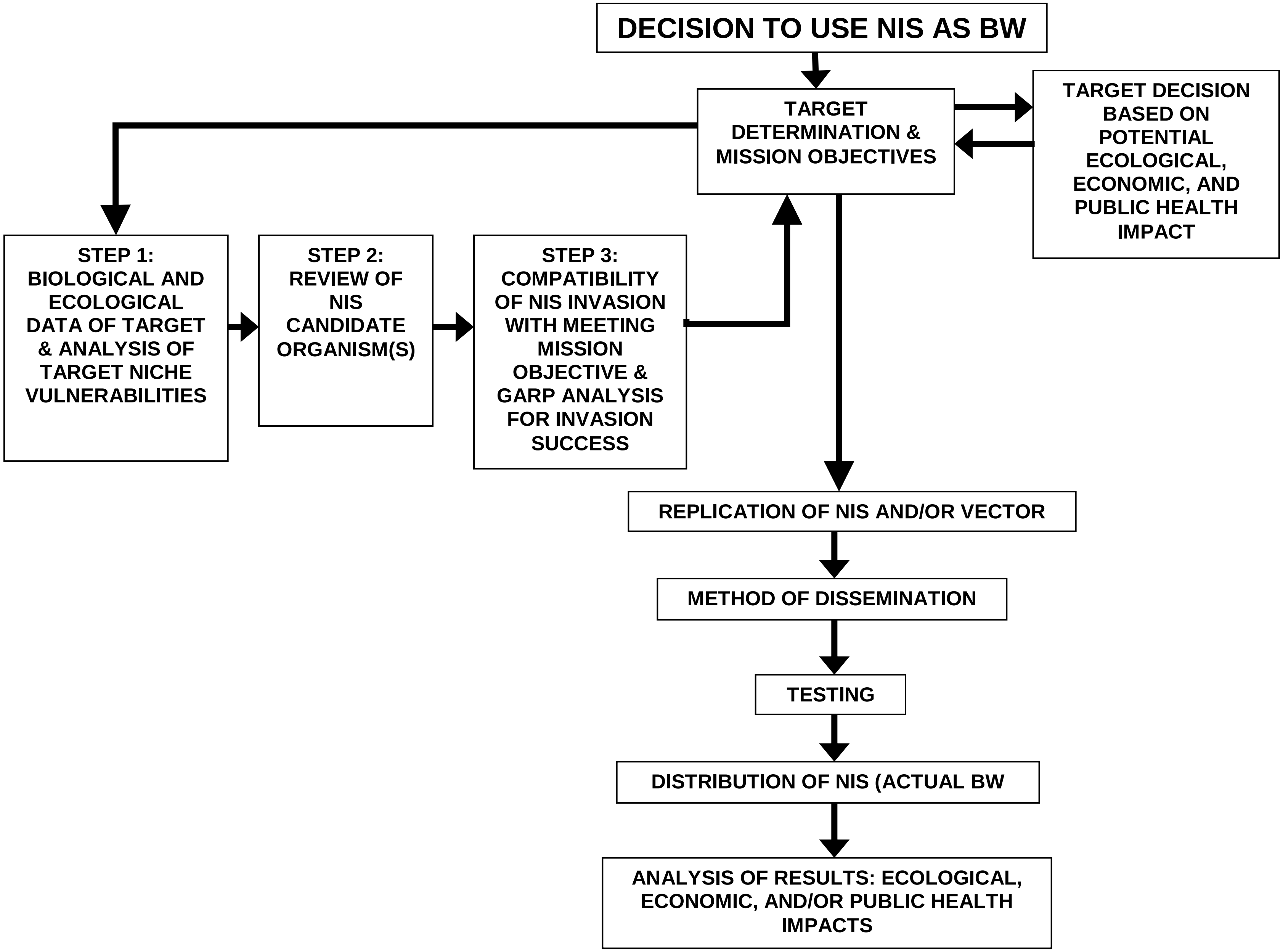
This chapter brings together the factors necessary to create an NIS BW attack. The applications of a NIS BW attack would depend on the target selected and the mission objective. The means to develop and analyze the attack are discussed. The users of NIS BW are varied (e.g. nation state, rouge state, criminal organization, terrorists, lone individual) and each aggressor will have various resources and limitations which would play a key role in whether to undertake a NIS BW attack. The uses of NIS for a BW attack can be subdivided into a single NIS agent with or without a vector carrier as well as a coordinated multiple agent attack directed to create an invasion meltdown of the targeted niche.

The chapter also considers the vulnerabilities of nations and niches as well as factors within specific niches that would enhance NIS invasion success. It is important to keep in mind that the effects of an NIS BW attack could affect not merely (human) public health, but damage ecosystems, agriculture, and economic targets.

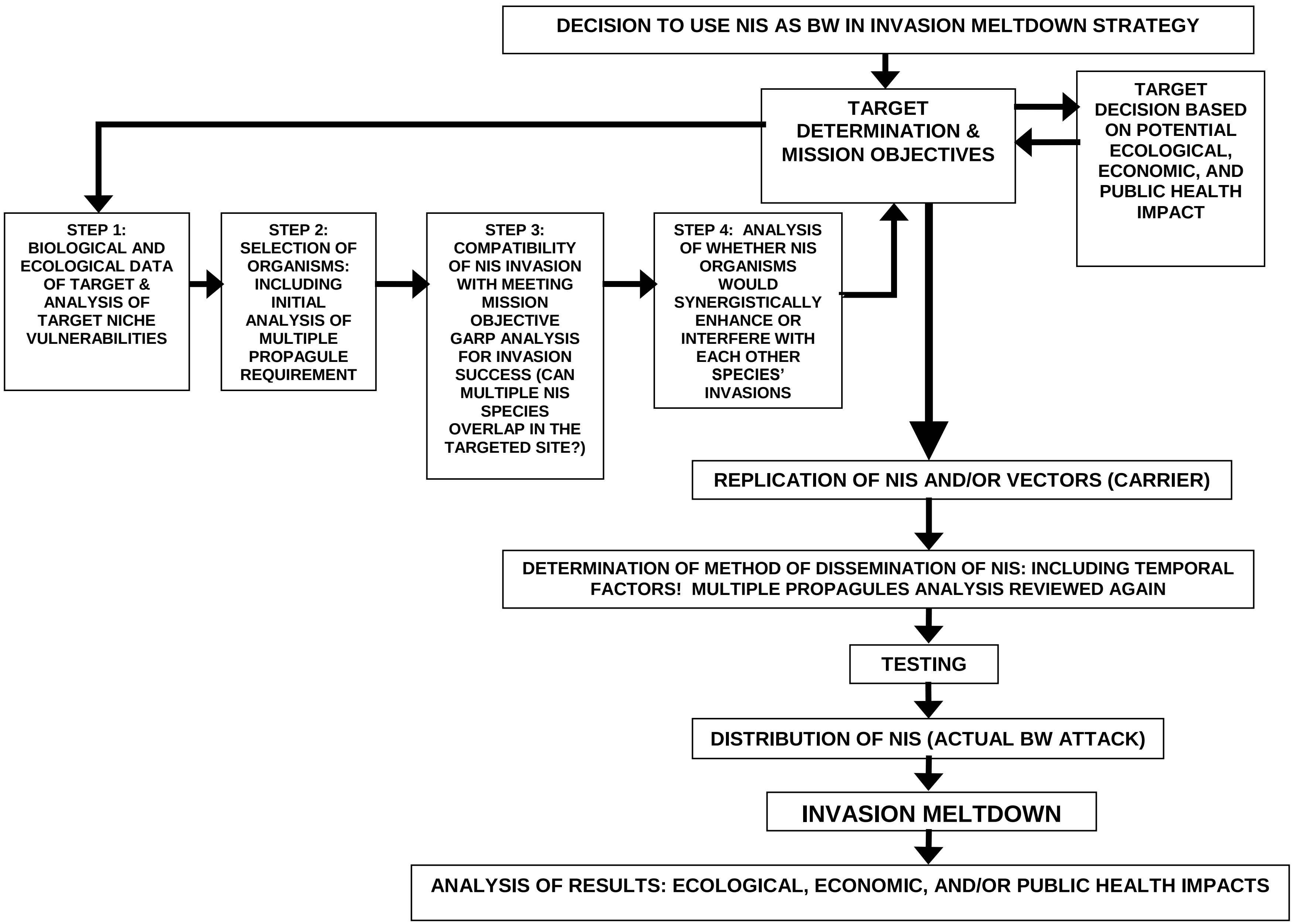
Although more data and research would be needed, this chapter provides a framework (supported by data from previous chapters) for how NIS could be used as a BW and how these NIS BW attacks could be applied. Hence, the present data and format supports the hypothesis that non-indigenous species (NIS) could be used as a biological weapon (BW).

The final section of this chapter explores four possible examples of NIS BW. Admittedly, all four scenarios are somewhat US-centric in their target selection and mission objectives. Each requires knowledge of the biogeographic and ecological variables for both the NIS candidates and target niche as well as the mission objectives (which include the potential ecological, economic, and public health impacts). Yet, by applying the same basic principles and operational procedures of NIS BW, any niche on the globe could be a target for a NIS BW attack depending on the mission objective.

FLOW CHART 1: PROCESS OF NIS BW ATTACK



FLOW CHART 2: MULTIPLE NIS SPECIES IN BW ATTACK TO ACHIEVE INVASION MELTDOWN



6. CITATIONS

1- Chalk, Peter. Hitting America's Soft Underbelly: The Potential Threat of Deliberate Biological Attacks Against the U.S. Agricultural and Food Industry. 1st ed. Santa Monica, CA: Rand Corporation, 2004.

2- Horn, Floyd P., Breeze, Roger G., Agriculture and Food Security. *In* Food and Agricultural Security: Guarding Against Natural Threats and Terrorist Attacks Affecting Health, National Food Supplies, and Agricultural Economics. Ed. Frazier, Thomas W. Richardson, Drew C., Vol. 894, Annals of the New York Academy of Sciences, New York, NY, 1999. 9-17.

3- Brown, Corrie, Economic Considerations of Agricultural Diseases. *In* Food and Agricultural Security: Guarding Against Natural Threats and Terrorist Attacks Affecting Health, National Food Supplies, and Agricultural Economics. Ed. Frazier, Thomas W. Richardson, Drew C., Vol. 894, Annals of the New York Academy of Sciences, New York, NY, 1999. 92-94.

4- Parker, Henry S., Agricultural Bioterrorism: A Federal Strategy to Meet the Threat. 1st ed. Honolulu, HI: University Press of the Pacific, 2004.

5- Hickson, Robert D., Infecting Soft Targets: Biological Weapons and Fabian Forms of Indirect Grand Strategy. *In* Food and Agricultural Security: Guarding Against Natural Threats and Terrorist Attacks Affecting Health, National Food Supplies, and Agricultural Economics. Ed. Frazier, Thomas W. Richardson, Drew C., Vol. 894, Annals of the New York Academy of Sciences, New York, NY, 1999. 108-117.

6- Sequeira, Ron, 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, National Food Supplies, and Agricultural Economics. Ed. Frazier, Thomas W. Richardson, Drew C., Vol. 894, Annals of the New York Academy of Sciences, New York, NY, 1999. 48-67.

7- Baskin, Yvonne. A plague of rats and rubber vines: The growing threat of species invasions. Washington, DC: Island Press, 2002.

8- Kadlec, Robert P. "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.

9- Lockwood, Jeffrey A. Six-Legged Soldiers: Using insects as Weapons of War. 1st. New York: Oxford University Press, 2009.

10- Whitby, Simon M., Biological Warfare against Crops. 1st ed. New York, NY : Palgrave, 2002.

11- Geissler, E., "Biological Warfare Activities in Germany, 1923-1945." Biological and Toxin Weapons: Research, Development and Use from the Middle Ages to 1945: No. 18-SIPRI Chemical & Biological Warfare Studies. ED. Zanders, J.P., Geissler, E., van Courtland Moon, J.E., New York, NY: Oxford University Press, 1999, 91-126.

12- Rogers, P., S. Whitby, and M. Dando. "Biological Warfare against Crops." Scientific American 280 (6), (June 1999): 70-75.

13- Ban, Jonathan. "Agricultural Biological Warfare: An Overview." Chemical and Biological Arms Control Institute Report, 9 (June 2000): 1-8.

14- Martin, Phyllis A.W., "A freeze-dried diet to test pathogens of Colorado potato beetle." *Biological Control*. 29. (2004): 109-114.

15- Gelman, Dale B., Bell, Robert A., ET AL. "Artificial diets for rearing the Colorado potato beetle, *Leptinotarsa decemlineata*." *Journal of Insect Science*. 1. (2001): 7-14.

16- Mack, Richard N. "Global Plant Dispersal, Naturalization, and Invasion: Pathways, Modes, and Circumstances." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 3-30.

17- Colautti, Robert, I., Niimi, Arthur, J., et al. "Spatial and Temporal Analysis of Transoceanic Shipping Vectors to the Great Lakes." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 227-246.

18- Fofonoff, Paul W., Ruiz, Gregory M., Steves, Brian, and Carlton, James T., "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*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 152-182.

19- Schumann, Gail L. Plant Diseases: Their Biology and Social Impact. St. Paul, MN: The American Phytopathological Society, 1991.

20- "Common Barberry *Berberis vulgaris*." *Weed of the Week*. USDA Forest Service-Invasive.org, 02 Oct 2006. Web. 2 Aug 2011. <http://www.invasive.org/weedcd/pdfs/wow/common-barberry.pdf>.

21- "Common barberry, European Barberry." *Invasive Plant Altas of New England*. University of Connecticut , 2009. Web. 2 Aug 2011. <http://nbii-nin.ciesin.columbia.edu/ipane/icat/browse.do?specieId=41>.

22- Kiziah, R.R.. "Assessment of the Emerging Biocruise Threat." The Gathering Biological Warfare Storm. ED. Davis, J.A., Schneider, B.R., Westport, CT: Praeger, 2004, 123-164.

23- Dickey, M.E. Lt. Col. USAF (2000).Biocruise: A Contemporary Threat. The Counterproliferation Papers, Future Warfare Series. 7, 1-33.

24- Petersburg, Ofer. "Israel's secret new weapon?: Israeli company uses nanotechnology to develop paint that makes planes disappear off radar ." *YNETNEWS.COM* 13 Jul 2010: Web. 31 Jul 2011. <http://www.ynetnews.com/Ext/Comp/ArticleLayout/CdaArticlePrintPreview/1,2506,L-3919257,00.html>.

25- "Israelis Use Nanotechnology to make Fighter Jets "Disappear"." *Nanovip.com*. Nanovip, LLC, 14 July 2010. Web. 31 Jul 2011. <http://www.nanovip.com/israelis-use-nanotechnology-to-make-fighter-jets-disappear.html>.

26- Stott, Michael. "Deadly new Russian weapon hides in shipping container." *Reuters.com* 26 Apr 2010: Web. 31 Jul 2011. <http://www.reuters.com/article/2010/04/26/us-russia-weapon-idUSTRE63P2XB20100426>.

27- "Club-K Container Missile System ." *www.concern-agat.com:* . Concern Morinformsystem-Agat, 2011. Web. 31 Jul 2011. <http://www.concern-agat.com/products/defense-products/81-concern-agat/189-club-k>.

28- Synovitz, Ron. "Russian Firm Denies 'Club-K' Missiles Could Be Used By Terrorists ." *Radio Free Europe/Radio Liberty.org* 26 Apr 2010: Web. 31 Jul 2011. <http://www.rferl.org/content/Russian_Firm_Denies_Club_K_Missiles_Could_Be_Used_By_Terrorists/2027728.html

29- "Russian Company Markets Hidden Cruise Missile System." *National Terror Alert Response Center*. Homeland Security News, 29 Apr 2010. Web. 31 Jul 2011. <http://www.nationalterroralert.com/2010/04/29/russian-company-markets-hidden-cruise-missile-system/>.

30-"Eco Tourism Industry: A brief introduction to Eco tourism Industry." *Economy Watch.com*. Stanley St Labs, 03 Aug 2011. Web. 3 Aug 2011. <http://www.economywatch.com/world-industries/tourism/eco.html>.

31- Simberloff, Daniel, and Betsy Von Holle. "Positive Interactions of Nonindigenous Species: Invasional Meltdown?." *Biological Invasions*. 1. (1999): 21-32.

32- Essl, Franz, and ET AL. "Socioeconomic legacy yields an invasion debt." *Proceedings of the National Academy of Science*. 108.1 (2011): 203-207.

33- Guillemin, Jeanne. "Bioterrorism and the Hazards of Secrecy: A History of Three Epidemic Cases." *Harvard Health Policy Review*. 4.1 (2003): 36-50.

34- Pheloung, Paul, "An Australian Perspective on the management of Pathways for Invasive Species." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 249-269.

35- Richardson, David M., Cambray, James A., Chapman, R. Arthur, et al., "Vectors and Pathways of Biological Invasions in South Africa-Past, Present, and Future." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 292-349.

36- Cavey, Joseph F., "Mitigating Introduction of Invasive Plant Pests in the United States." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 350-361.

37- Reaser, Jamie K., Yeager, Brooks B., Phifer, Paul R., et al, "Environmental Diplomacy and the Global Movement of invasive Alien Species: A U.S. Perspective." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 362-381.

38-*Predicting Invasions of Nonindigenous Plants and Plants Pests*. National Research Council. Washington, DC: National Academy Press, 2002.

39-Oppel, Steffen, Brent M. Beaven, and ET AL. "Eradication of Invasive Mammals on Islands inhabited by humans and Domestic Animals." *Conservation Biology*. 25.2 (2010): 232-240.

40- Pravecek, T.L., Davis, J.A., "Bio-Defense Now: 56 Suggestions for Immediate Improvements-Final Report 85% Biological Defense Project." USAF Counterproliferation Center. May 2005. Air University, Maxwell Air Force Base, Alabama. 27 Jul 2008 <http://www.au.af.mil/au/awc/awcgate/cpc-pubs/pravecek.pdf>.

41- Goku, K. "Biosecurity measures to prevent the incursion of invasive alien species in Japan and to mitigate their impact." *Rev. sci. tech. Off. int. Epiz.*. 29.2 (2010): 299-310.

42- Orr, R.L., Cohen, S.D., and Griffin, R.L., *Generic Non-Indigenous Pest Risk Assessment Process*. Beltsville, MD: U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 1993.

43- Orr, Richard, and et al. "Report to the Aquatic Nuisance Species Task Force." *Generic Nonindigenous Aquatic Organisms Risk Analysis Review Process*. Bethesda, MD: Aquatic Nuisance Species Task Force, 1996.

44- Hansen, Malin J., and Clevenger, Anthony P. "The influence of disturbance and habitat on the presence of non-native plant species along transport corridors." *Biological Conservation*. 125.2 (2005): 249-259.

45- Mack, Michelle C., and D'Antonio, Carla M., "Impacts of biological invasions on disturbance regimes." *Trends in Ecology & Evolution*. 13.5 (1998): 195-198.

46- With, Kimberly A. "Assessing the Risk of Invasive Spread in Fragmented Landscapes." *Risk Analysis*. 24.4 (2004): 803-815.

47- Dudley, Joseph P., and Michael H. Woodford. "Bioweapons, Biodiversity, and Ecocide: Potential Effects of Biological Weapons on Biological Diversity." *Bioscience*. 52.7 (2002): 582-592.

48- Kolar, Cynthia S., and David M. Lodge. "Progress in invasion biology: predicting invaders." *TRENDS in Ecology & Evolution*. 16.4 (2001): 199-204.

49- Luby, Stephen P., Emily S. Gurley, and M. Jahangir Hossain. "Transmission of Human Infection with Nipah Virus." *Clinical Infectious Diseases*. 49. (2009): 1743-1748.

50- Luby, Stephen P., M. Jahangir Hossain, and ET AL. "Recurrent Zoonotic Transmission of Nipah Virus into Humans, Bangladesh, 2001-2007." *Emerging Infectious Diseases*. 15.8 (2009): 1229-1235.

51- Daniels, P.W., K. Halpin, A. Hyatt, and D. Middleton. "Infection and Disease in Reservoir and Spillover Hosts: Determinants of Pathogen Emergence ." *Current Topics in Microbiology and Immunology*. 315. (2007): 113-131.

52- Weingartl, Han M., Yohannes Berhane, and Markus Czub. "Animal models of Henipavirus infection: A review." The *Veterinary Journal*. 181. (2009): 211-220.

53- Weingartl, Hana, Stefanie Czub, and ET AL. "Invasion of the Central Nervous System in a Porcine Host by Nipah Virus." *Journal of Virology*. 79.12 (2005): 7528-7534.

54-"Bioterrorism Agents/Diseases." *Bioterrorism Agents/Diseases*. Centers for Disease Control and Prevention, 04 Aug 2011. Web. 4 Aug 2011. <http://www.bt.cdc.gov/agent/agentlist-category.asp#c>.

55- Dell'Amore, Christine. "Wild Pig Explosion May Spread Disease to Humans." *National Geographic News*. National Geographic Society, 02 May 2011. Web. 4 Aug 2011.

<http://news.nationalgeographic.com/news/2011/05/110502-wild-pigs-parasites-animals-pork-science-health-nation/>.

56- Salway, Mary Kay. "Feral Pigs." *Wildlife Management* . Wisconsin Department of Natural Resources, 10 Mar 2009. Web. 4 Aug 2011. <http://dnr.wi.gov/org/land/wildlife/publ/wlnotebook/pig.htm>.

57- "Feral/Wild Pigs: Potential Problems for farmers and Hunters." *Agriculture Information Bulletin No. 799*. USDA, Animal and Plant Health Inspection Service, Oct 2006. Web. 4 Aug 2011. <http://www.aphis.usda.gov/publications/wildlife_damage/content/printable_version/feral%20pigs.pdf>.

58- Sandfoss, Mark, Christopher DePerno, and ET AL. "Prevalence of Antibody to *Toxoplasma Gondii* and *Trichinella* spp. in Feral Pigs (*Sus scrofa*) of Eastern North Carolina." *Journal of Wildlife Diseases*. 47.2 (2011): 338-343.

59- Morthland, John. "A Plague of Pigs in Texas." *Smithsonian.com*. Smithsonian Media, Jan 2011. Web. 4 Aug 2011. <http://www.smithsonianmag.com/science-nature/A-Plague-of-Pigs-in-Texas.html?c=y&page=1>.

60- Musselman, Lytton J. "The Biology of *Striga*, *Orobanche*, and Other Root-Parasitic Weeds." *Annu. Rev. Phytopathology*. 18. (1980): 463-489.

61- Scholes, Julie D., and Malcolm C. Press. "*Striga* infestation of cereal crops-an unsolved problem in resource limited agriculture." *Current Opinion in Plant Biology*. 11. (2008): 180-186.

62- Nour, Jane, Malcolm Press, and ET AL. "Africa in the grip of witchweed." *New Scientist*. 109. (1986): 44-48.

63- Mohamed, Kamal I., Monica Papes, and ET AL. "Global Invasive Potential of 10 Parasitic Witchweeds and Related *Orobanchaceae*." *Ambio*. 35.6 (2006): 281-288.

64- Mohamed, K. I., J. F. Bolin, L. J. Musselman, and A. T. Peterson., Genetic diversity in Striga and implications for control and future distributions. In " *International Symposium on Integrating New Technologies for Striga Control: Towards Ending the Witch-hunt", Addis Ababa, Ethiopia, November 5-11, 2006*. Ed. J. Gressel and G. Ejeta. Singapore: World Scientific Publishing, 2006. Pages 71-84.

65- Eplee, Robert E. "Striga's Status as a Plant Parasite in the United States." *Plant Disease*. 65.12 (1981): 951-954.

66- "Tropical Bont Tick ." *USDA-Animal and Plant Inspection Service (APHIS)*. United States Department of Agriculture, 28 Nov 2006. Web. 5 Aug 2011. <http://www.aphis.usda.gov/international_safeguarding/tropical_bont_tick.shtml>.

67- "TROPICAL BONT TICK." *International Food Safety Consultancy*. International Food Safety Consultancy, Web. 5 Aug 2011. <http://www.international-food-safety.com/pdf/tropicalbonttick.pdf>.

68- Corn, J. L., et al, Potential Impact of Wildlife on the Tropical Bont Tick Eradication Program in the Caribbean. *In* Vector-Borne Pathogens: International Trade and Tropical Animal Diseases. Eds. Camus, E., House, J.A., Uilenberg, G., Vol. 791, Annals of the New York Academy of Sciences, New York, NY, 1996. 77-84.

69- Burridge, M. J. et al, 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. Eds. Gibbs, E. P. J., Bokma, B. H., Vol. 969, Annals of the New York Academy of Sciences, New York, NY, 2002. 269-274.

70- Kock, Nancy D. "Heartwater." *Infectious Diseases of Wild Animals, 3rd Edition*. Ed. Elizabeth S. Williams, Ian K. Barker. Ames, Iowa: Iowa State University Press, 2001, 477-480.

71- Uilenberg, G., Progress and Priorities in Research on Heartwater. *In* Vector-Borne Pathogens: International Trade and Tropical Animal Diseases. Eds. Camus, E., House, J.A., Uilenberg, G., Vol. 791, Annals of the New York Academy of Sciences, New York, NY, 1996. 1-16.

72- Burridge, M.J., 1997. Heartwater: an increasingly serious threat to the livestock and deer populations of the United States. *In* Proceedings of the 101st Annual Meeting of the United States Animal Health Association, Spectrum Press, Richmond, VA, 582-597.

73- "HEARTWATER - COWDRIOSIS." *World Organization for Animal Health*. OIE Scientific and Technical Department, Oct 2009. Web. 6 Aug 2011. <http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/HEARTWATER_FINAL.pdf>.

74 - Allsopp, M.T.E.P., Louw, M., Meyer, E.C., Ehrlichia ruminantium: An Emerging human Pathogen? *In* Rickettsioses: From Genome to Proteome, Pathobiology, and Rickettsiae as an International Threat. Eds. Hechemy, K.E., Oteo, J.A., Raoult, D.A., Silverman, D.J., Blanco, J.R., Vol. 1063, Annals of the New York Academy of Sciences, New York, NY, 2005. 358-360.

75-"Record Corn Crop will Boost Biofuel Market." *Market Research Analyst.com*. Market Research Analyst, 14 May 2011. Web. 7 Aug 2011. <http://www.marketresearchanalyst.com/2011/05/14/record-corn-crop-will-boost-biofuel-market/>.

76- Pillai, P.K., R.D. Willcoxson, and R.D. Raychaudhuri. "Production of teliospores by some races of Puccinia graminis f. sp. tritici in detached wheat and barley leaves ." *Mycopathologia* . 64.2 (1978): 101-103.

77- Madden, Laurence V., and Frank Van den Bosch. "A Population-Dynamics Approach to Assess the Threat of Plant Pathogens as Biological Weapons against Annual Crops." *BioScience*. 52.1 (2002): 65-74.

78- Klinkowski, M. "Catastrophic plant diseases." Annual Reviews in Phytopathology 8(1970): 37-60.

79- Amri, A., A. Yahyaoui, and ET AL. "Wheat rust population dynamics and its implication on global wheat production: Case study of Ug99." *Institutional analysis of collective action arrangements for sustainability* . Biodiversity and Reflexive governance (BIOGOV), 25 Mar 2009. Web. 4 Aug 2011.
<http://biogov.cpdr.ucl.ac.be/Biov_FAO_Workshops/Amri%20Ahmed.pdf>

80- Food and Agricultural Organization of the United Nations. "Wheat Killer Detected In Iran: Dangerous Fungus On The Move From East Africa To The Middle East." *ScienceDaily*, 17 Mar. 2008. Web. 4 Aug. 2011.

CHAPTER 8. METHODS TO DISCERN OR DETECT A DELIBERATE ATTACK

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CHAPTER 8: METHODS TO DISCERN OR DETECT A DELIBERATE ATTACK

1. INTRODUCTION

As chapter 7 and the data from the other chapters support the hypothesis that Non-Indigenous Species (NIS) can be used as a Biological Weapon (BW), the question arises as to how could a deliberate release 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, 2).

One example of a possible NIS BW attack in the past is the case of "The Breeders". According to Horn et al (3) and Root-Bernstein (4), In 1989, a group calling itself "The Breeders" announced that they had bred and released Mediterranean fruit flies (*Ceratitis capitata Weidemann*) to protest the use of pesticides in the southern California area. This was during a decade long eradication program run by combined USDA APHIS and California Dept. of Food and Agriculture (CDFA) to rid the state of California of the NIS Mediterranean fruit fly (aka Medfly). The Medfly is a native of Africa, but had become an NIS in Southern Europe, Australia, and many South America countries (5, 6, and 7). The Medfly was viewed as a major threat to California agriculture due to its wide host range (recorded to be over 300 cultivated and wild fruits), including apple, avocado, citrus fruits, and tomatoes (5, 6, and 7). During the 1980's, traps would be used to monitor for the presence of the Medflies with subsequent evening spraying of the organophosphate pesticide, Malathion, where traps caught Medflies (8). This eradication process was followed up by large scale releases of sterile male Medflies to disrupt the insect's reproductive cycle. To the present date, eradication efforts have continued to limit the spread of the species.

The Breeders appeared to demand the end of all spraying in the state of California (8). During this time period, unusual appearances of Medflies appeared in traps in areas previously sprayed and believed to be Medfly free (8). Later, a US Department of Agriculture (USDA) study identified peculiar patterns of Mediterranean fruit fly (Medfly) infestations especially in new and strange places where the fruit fly would not likely appear. A review panel which included USDA scientists, concluded that someone or group was in fact breeding and releasing Medfly larvae. Follow up attempts to communicate with the group yielded no criminal leads and no one to date has come forth or been apprehended over the incident, which Lockwood referred to as "ecoterrorism" (4, 8, and 9).

2. 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) (29). To rule out accidental release, investigators would need to examine (depending on the characteristics of the target niche) if any commercial 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 (13); the introduction of the Asian Longhorned Beetle (*Anoplophora glabripennis*) via solid wood packing materials from China (30); the introduction of the zebra mussel (*Dreissena polymorpa*) into the North American Great Lakes from shipping ballast water (14); 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 (15).

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 (10), the accidental release of the spiny-tailed black iguana (*Ctenosaura similis*) as exotic pets onto Gasparilla Island (Florida) (11), or the escape of Gambian Rats (*Cricetomys gambianus*) from an exotic pet breeder into Grassy Key, Florida (12).

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 (18, 20). 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 (16), 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 (17), 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 (18, 19), 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 (20, 21), and the wind transport of fungal spores of the NIS Tobacco Blue Mold (*Peronospora tabacina Adam*) across the US (22, 23).

Pearson (2) 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" (2). These points can be applied in a similar fashion for NIS BW if the NIS is compared to the pathogen (host agent) and 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 " from a BW pathogen similar to the term "invasion" by 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. (Source 2-pg. 13).

Sequeira (1) 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):

1. Use of non-traditional pathways; if evidence of delivery is via smuggling or aerial delivery.
2. Increase of the probability of survival of the pest in transit; NIS BW may require careful culturing and storage prior to distribution.
3. 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..
4. Use of highly virulent strains; strains could also be genetically engineered to enhance survival (see below Black Biology).
5. High rates of inoculum; this concept follow with the propagule pressure concept-the more propagules-either in single or multiple dispersals enhances the probability of successful NIS invasion and colonization.
6. Introduction into remote areas; remote areas favor the time lag necessary for colonization and reduce the risk of early detection and eradication efforts.
7. Targeting of susceptible production areas; as reviewed in Chapter 7, the best target niches would have biogeographic factors favoring invasion and colonization.
8. Targeting of susceptible natural environments; comments similar to Chapter 7, except natural environments may also be determined by GARP or similar ENM analysis.

9. Release of multiple species simultaneously; this follow with an invasion meltdown approach where several different organisms enhance the impact on the target niche and result in quicker alteration of the target niche.
10. 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. (SOURCE 1-PG 49-50)

Sequeira also notes that the globalization of the economy has already taxed the existing USDA structures and resources, especially APHIS (1). Hence, APHIS might not be prepared to handle an NIS BW attack as described in Chapter 7. 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 (1).

Asner et al (31) 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 (31). 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.

3. 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, Petersen notes (2) 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 also be monitoring for evidence of Black Biology 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.

4. EVIDENCE OF BLACK BIOLOGY

As previously described in Chapter 3, black biology would be the defined as the use of recombinant DNA technology towards the development of Biological Weapons. 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) 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), 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.

5. GOVERNMENT INVESTIGATION INTO ENTRY VIA SMUGGLED OR IMPORTED PETS OR FOOD

As described by Kadlec (24), 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. Kaldec 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 (24). A National Research Council report (25) 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 as 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 (25). 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 (25). 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 (25).

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, SEE 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.

6. 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) (26).

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. “(26).

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.” (26).

Hence, this reaffirmation states that genetically altered organisms for BW -even NIS BW- would be in violation of the BTWC (26).

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." (27)

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". (27).

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 (28), 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." (28)

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 (28).

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.

7. SUMMARY

As the previous chapters built up data and strategies to support the hypothesis that NIS could be used as a biological weapon, the challenge in this chapter was focused on how to discern that an actual NIS invasion is a BW event. Although the 1989 Breeder's bioterrorist event was never solved, the challenge will be how to discern accidental NIS invasions from deliberate NIS BW attacks in the future.

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 "hitched a ride' on migrating organisms like birds or insects.

As this approach occurs, it must be noted that several researchers using established BW protocol for analysis of unusual outbreaks provided 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 was genetically engineered to improve its invasiveness or colonization, this should raise a “red flag” to the intelligence and law enforcement network as well as 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.

Finally, 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).

TABLE 1-NIS DISPERSAL MODE ANALYSIS: A STRATEGY TO RULE OUT ACCIDENTAL FROM DELIBERATE INTRODUCTION OF NIS		
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

NOTE: If questions #1, #2, #3 alone are yes, then some investigation required-but if in combination with yes to questions #4 or #6, then further concerned investigation is warranted. #7- the value of the NIS in the pathway usually would be presumed to serve little "reasonable" purpose, especially if it is traveling on a "smuggled" pathway, instead of a licit pathway. Risk would be elevated if the NIS in #7 was transported in large numbers (propagules) as in question #6.

9. CITATIONS

1- Sequeira, Ron, 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, National Food Supplies, and Agricultural Economics. Ed. Frazier, Thomas W. Richardson, Drew C., Vol. 894, Annals of the New York Academy of Sciences, New York, NY, 1999. 48-67.

2- Pearson, Graham S,. "The Importance of Distinguishing Between Natural and Other Outbreaks of Disease." *Scientific and Technical Means of Distinguishing Between Natural and Other Outbreaks of Disease*. Ed. Malcolm Dando, Graham Pearson and Bohumir Kriz. Dordrecht, the Netherlands: Kluwer Academic Publishers, 2001. 1-20.

3- Horn, Floyd P., Breeze, Roger G., Agriculture and Food Security. *In* Food and Agricultural Security: Guarding Against Natural Threats and Terrorist Attacks Affecting Health, National Food Supplies, and Agricultural Economics. Ed. Frazier, Thomas W. Richardson, Drew C., Vol. 894, Annals of the New York Academy of Sciences, New York, NY, 1999. 9-17.

4- Root-Bernstein, Robert S., "Infectious Terrorism." The Atlantic Monthly vol. 267, No. 5 (May 1991): 44-50.

5- "Mediterranean Fruit Fly Pest Profile." *California Dept. of Food and Agriculture*. State of California, 2011. Web. 15 Aug 2011. <http://www.cdfa.ca.gov/phpps/pdep/target_pest_disease_profiles/mediterranean_ff_profile.html>.

6- "Mediterranean Fruit Fly Fact Sheet." *CDFA.CA.GOV*. California Dept. of Food and Agriculture, 23 Oct 2008. Web. 15 Aug 2011. <http://www.cdfa.ca.gov/phpps/factsheets/MedFlyFactSheet.pdf>.

7- "Mediterranean fruit fly, Ceratitis capitata (Wiedemann)." *UNIVERSITY of FLORIDA ENTOMOLOGY and NEMATOLOGY DEPARTMENT*. University of Florida, Sept 2010. Web. 15 Aug 2011. <http://entnemdept.ufl.edu/creatures/fruit/mediterranean_fruit_fly.htm>.

8- Lockwood, Jeffrey A. Six-Legged Soldiers: Using Insects as Weapons of War. New York: Oxford University Press, 2009. 234-240.

9- Lockwood, Jeffrey. "Could ecoterrorists let slip the bugs of war?." *Sunday Times* 02, Feb, 2009: Web. 15 Aug 2011. <http://www.timesonline.co.uk/tol/comment/columnists/guest_contributors/article5634450.ece>.

10- Meinesz, Alexandre, and Translated by Daniel Simberloff. Killer Algae. Chicago: University of Chicago Press, 1999.

11- Stoddard, Grant. "The Lizard King." *Hemispheres Magazine*. June 2011: 90-95.

12- Perry, Neil D., and ET AL. "New Invasive Species in Southern Florida: Gambian Rat (*Cricetomys Gambianus*)." *Journal of Mammalogy*. 87.2 (2006): 262-264.

13- Palm, Mary, E., Rossman, Amy Y. "Invasion Pathways of Terrestrial Plant-Inhabiting Fungi." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 31-43.

14- Fofonoff, Paul W., Ruiz, Gregory M., Steves, Brian, and Carlton, James T. "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*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 152-182.

15- Kiritani, Keizi and Yamamura, Kohji "Exotic Insects and their Pathways for Invasion." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 44-67.

16- Witmer, Gary W., Nathan P. Snow, and Patrick W. Burke. "Potential Attractants for Detecting and Removing Invading Gambian Giant Pouched Rats (Cricetomys gambianus)." *Pest Management Science*. 66. (2010): 412-416.

17- Burridge, M. J. et al, 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. Eds. Gibbs, E. P. J., Bokma, B. H., Vol. 969, Annals of the New York Academy of Sciences, New York, NY, 2002. 269-274.

18- Irey, M., Gottwald, T. R., Graham, J. H., Riley, T. D., and 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. Online. Plant Health Progress doi:10.1094/PHP-2006-0822-01-RS.

19- Gottwald, T. R., and 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. Online. Plant Health Progress doi:10.1094/PHP-2007-0405-01-RS.

20- Amri, A., A. Yahyaoui, and ET AL. "Wheat rust population dynamics and its implication on global wheat production: Case study of Ug99." *Institutional analysis of collective action arrangements for sustainability* . Biodiversity and Reflexive governance (BIOGOV), 25 Mar 2009. Web. 4 Aug 2011.
<http://biogov.cpdr.ucl.ac.be/Biov_FAO_Workshops/Amri%20Ahmed.pdf>

21- Food and Agricultural Organization of the United Nations. "Wheat Killer Detected In Iran: Dangerous Fungus On The Move From East Africa To The Middle East." *ScienceDaily*, 17 Mar. 2008. Web. 4 Aug. 2011.

22- Main, C.E., Keever, T., Holmes, G.J. and Davis, J.M. 2001. Forecasting Long-Range Transport of Downy Mildew Spores and Plant Disease Epidemics. APSnet Features. Online. doi: 10.1094/APSnetFeature-2001-0501

23- Main, C. E., and Davis, J. M. 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.

24-Kadlec, Robert P. "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.

25- *Predicting Invasions of Nonindigenous Plants and Plants Pests*. National Research Council. Washington, DC: National Academy Press, 2002.

26- "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, 2009. Web. 17 Aug 2011. <http://www.unog.ch/80256EDD006B8954/(httpAssets)/66E5525B50871CAEC1257188003BDDD6/$file/BWC_Text_Additional_Understandings.pdf>.

27- "Convention on the Prohibition of Military or Any Other Hostile Use of Environmental Modification Techniques ." *United States Department of State*. U.S. Department of State, 10 Jul 2008. Web. 17 Aug 2011. <http://web.archive.org/web/20080710174615/http://www.state.gov/t/ac/trt/4783.htm>.

28- "Protocol Additional to the Geneva Conventions of 12 August 1949, and relating to the Protection of Victims of International Armed Conflicts (Protocol I), 8 June 1977." *International Humanitarian Law - Treaties & Documents*. International Committee of the Red Cross, 2005. Web. 17 Aug 2011. <http://www.icrc.org/ihl.nsf/FULL/470?OpenDocument>.

29- Mack, Richard N. "Global Plant Dispersal, Naturalization, and Invasion: Pathways, Modes, and Circumstances." *Invasive Species: Vectors and Management Strategies*. Ed: Gregory M. Ruiz, James T. Carlton. Washington, DC: Island Press, 2003, 3-30.

30- Meyer, Dan A. "SPECIAL REPORT: Asian Longhorned Beetle." *The Entomology and Forest Resources Digital Information Work Group*. Invasive.org, Nov 1998. Web. 27 Aug 2011. <http://www.invasive.org/publications/98025.pdf>.

31- Asner, Gregory P., R. Flint Hughes, and ET AL. "Invasive plants transform the three-dimensional structure of rain forests." *Proceedings of the National Academy of Science*. 105.11 (2008): 4519-4523.

CHAPTER 9. RECOMMENDATIONS FOR COUNTERSTRATEGIES

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CHAPTER 9. RECOMMENDATIONS FOR COUNTERSTRATEGIES

1. INTRODUCTION

At present, there are limitations for counterstrategies to a Non-Indigenous Species based Biological Warfare (NIS BW) attack. The intent of this chapter 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 in Chapter 3 by Lebeda (1) 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 of treatments would include eradication and biocontrol measure for NIS organisms causing non-human or non-agricultural damage (e.g. ecological damage). This of course, takes into consideration that the NIS agents that do cause morbidity or mortality to humans, livestock, crops, already have drugs, vaccines, or other counter agents (e.g. pesticides, herbicides, antifungal compounds, etc.) ready to respond to a NIS BW attack.

Yet, in the world of bioterrorism, asymmetrical warfare tactics as described in Chapter 3 by McKenzie (2), would favor use of 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.

2. BORDER CONTAINMENT AND CONTROL

Sequeira (3) 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 (3) notes that APHIS centers must evolve and communicate better with sister agencies (e.g. 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 National Research Council (NRC) in 2002 made a series of recommendations to enhance the scientific basis for predicting invasive potential of plants and plant pests (4). 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 (4, pg 143-145) 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 become peer-reviewed; and update the process to capture new information and provide for improvements in expert judgment (4, pg 143-145). 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 (4, pg 143-145). 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 section 5 below).

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 previous stated in Chapter 7, developing nations would have a unique level of vulnerability to NIS BW attack due to their limited resources and diminished economic vigor.

3. 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 (4) offer several areas of NIS research that would help in NIS BW counterstrategies. The report (4, pg.147-150) 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 (4, pg.147-150). 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 (4, pg.147-150). It is the species attacking US native plants that must undergo a risk analysis for potential arrival as an NIS to US soil. Also, 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. Also, 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).

4. EXPANSION OF NIS DATABASES

Databases on NIS do presently exist. Two of the most notable are the National Invasive Species Information Center (NISIC) (5) and the Global Invasive Species Database (GISD) (6).

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 (4, pg 143-145) 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 (4, pg 145-147) 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 (4, pg 145-147) 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.

5. 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 (7) 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 (7). 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 (8, 9, 10, and 11), 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 (12). Linder, Huang and team (12) 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 (14) 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 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 (14). 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 (13) notes that the genotyping of pathogens would aid infectious disease specialists and HUMINT in the identification of BW agents as well as genetically engineered BW agents (13). 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 (15) 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 (15). 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 (16) 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*) (16). The authors state that the DNA signatures would be important in the detection of genetically altered bacteria in environmental samples (16). 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.

6. SUMMARY

The purpose of this chapter was to suggest where further research and expansion of techniques could 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. Furthermore, 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 possibly 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.

7. CITATIONS:

1- Lebeda, F.J., "Deterrence of Biological and Chemical Warfare: A Review of Policy Options." Military Medicine 162, No. 3(1997): 156-161.

2- McKenzie, Kenneth F. "The Revenge of the Melians: Asymmetric Threats and the Next QDR: McNair Paper 62." *Air War College, MAXWELL AIR FORCE BASE, MONTGOMERY, ALABAMA* . INSTITUTE FOR NATIONAL STRATEGIC STUDIES NATIONAL DEFENSE UNIVERSITY, 2000. Web. 28 Aug 2011. <http://www.au.af.mil/au/awc/awcgate/ndu/mcnair62.pdf>.

3- Sequeira, Ron, 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, National Food Supplies, and Agricultural Economics. Ed. Frazier, Thomas W. Richardson, Drew C., Vol. 894, Annals of the New York Academy of Sciences, New York, NY, 1999. 48-67.

4- *Predicting Invasions of Nonindigenous Plants and Plants Pests*. National Research Council. Washington, DC: National Academy Press, 2002.

5- "National Invasive Species Information Center (NISIC)." *National Agricultural Library*. United States Department of Agriculture, 22 Aug 2011. Web. 23 Aug 2011. <http://www.invasivespeciesinfo.gov/>.

6- "Global Invasive Species Database (GISD)." *Invasive Species Specialist Group (ISSG)*. IUCN Species Survival Commission, 2011. Web. 23 Aug 2011. <http://www.issg.org/database/welcome/>.

7- Scorza, Ralph. "Ecology and Genetics of Exotics." In *Exotics, Pests, Plants, and North American Agriculture*. Eds. C.L. Wilson and C.L. Graham. New York, NY: Academic Press, 1983. 219-238.

8- Guzman, Eduard, Antoni Romeu, and Santiago Garcia-Vallve. "Completely sequenced genomes of pathogenic bacteria: A review." *Enferm Infecc Microbiol Clin*. 26.2 (2008): 88-98.

9- Seshadri, Rekha, Read, Timothy D., ET AL. "Genomic Efforts With Biodefense Pathogens." In *Biological Weapons Defense: Infectious Diseases and Counterbioterrorism*. Ed. Luther E. Lindler, Frank J. Lebeda, George W. Korch: Totowa, NJ, Humana Press. 2005. 417-433.

10- Andersson, Siv G.E., Forsman, Mats, ET AL. "Genomics for Biodefense: Exploiting the *Francisella tularensis* Genome Sequence." In *Biological Weapons Defense: Infectious Diseases and Counterbioterrorism*. Ed. Luther E. Lindler, Frank J. Lebeda, George W. Korch: Totowa, NJ, Humana Press. 2005. 435-452.

11- Linder, Luther E. "*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. 2005. 481-505.

12- Linder, Luther E., Huang, Xiao-Zhe, and ET AL. "Genetic Fingerprinting of Biodefense Pathogens for Epidemiology and Forensic Investigation." In *Biological Weapons Defense: Infectious Diseases and Counterbioterrorism*. Ed. Luther E. Lindler, Frank J. Lebeda, George W. Korch: Totowa, NJ, Humana Press. 2005. 453-480.

13- Linder, Luther E., Choffnes, Eileen, and Korch, George W. "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. 2005. 351-360.

14- Schaad, Norman W., D. Frederick Reid, and ET AL . "Advances in Molecular-Based Diagnostics in Meeting Crop Biosecurity and Phytosanitary Issues." *Annual Review of Phytopathology*. 41. (2003): 305-324.

15- Black, John Logan. "Genome Projects and Gene Therapy: Gateways to Next Generation Biological Weapons." *Military Medicine*. 168.11 (2003): 864-871.

16- Allen, Jonathan E., Shea N. Gardner, and Tom R. Slezak. "DNA signatures for detecting genetic engineering in bacteria." *Genome Biology*. 9.3 (2008): doi: 10.1186/gb-2008-9-3-r56.

CHAPTER 10. SUMMARY

CHAPTER 10: SUMMARY

1. SUMMARY

The purpose of this dissertation was to explore the hypothesis that non-indigenous species (NIS) could be used as a form of biological weapon (BW). The data supports the hypothesis that NIS could be used as a form of BW. Couch defines a biological weapons attack as “the intentional use by the enemy, of live agent or toxins to cause death and disease among citizens, animals, and plants" (1). NIS are non-native organisms that inflict damage to naïve niches once the NIS organism has been introduced into niche. NIS invasions could act at a strategic or even tactical level to incite economic damage; disease outbreaks to humans, livestock, or agricultural crops; cause ecological damage to niches ; and eventually create social unrest and panic in populations. NIS BW is similar to conventional BW weapons in that it can be a tool of asymmetrical warfare that leverages a bioterrorist’s weaknesses in warfare (e.g. inferior tactical or operational strength) against a nation’s vulnerabilities and hence achieves a disproportionate effect on the targeted society (2). As a result, the effects on the target niche could further result in disproportionate effects on the society such as social chaos, psychological fear, ecological destruction, and economic damage.

The methods to predict success of an NIS BW include a review of the NIS history; use of ecological niche modeling (ENM) tools such as GARP to determine target niche compatibility and NIS BW success; and calculate and reproduce the propagule needs of the NIS necessary to achieve a successful invasion. It must be noted that, depending on the NIS organism used, even failed invasions may elicit terror in the public, which is the prime motivation of a bioterrorist attack.

The strategies of attack include targeting disrupted niches including; sites of monoculture (e.g. agricultural crops, biofuel crops, etc.); sites disrupted by human activity (e.g. war zone, deforestation, erosion, pollution, roadway construction); or damaged by wildfires, climate change, or limited genetic diversity. Also, the attack must consider not merely the target site, but the NIS organism and the time and cultivation necessary to achieve the mission objectives (e.g. destruction of food crops, biofuel crops, disease outbreaks, etc.). Further strategies for the attack include review of the delivery of the NIS whether that process is hand distribution, aerial dispersal or high tech dispersal (e.g. Biocruise) as well as whether the biosecurity measures must be evaded during or prior to dispersal.

The lag time until invasion or colonization depends on the NIS, but similar to a conventional BW attack, the lag time provides the attacker with “plausible deniability” for responsibility of the attack as well as time to escape from the target niche and/or NIS dispersal site.

The risk factors that could make a target more vulnerable to attack include poor biosecurity (e.g. poor border control), limited resources to monitor and eradicate NIS invasions, fragile ecosystems, limited biodiversity of a target niche, nations dependent on single staple food crops, and poor communication between scientific authorities and government law makers and regulatory agencies.

The methods to detect an NIS BW attack as well as discern an accidental introduction from a deliberate attack are diverse. 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 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.

In any case of NIS BW, it clearly will be considered a violation of the Biological and Toxin Weapons Convention (BTWC) as the NIS is considered a biological agent used for hostile purposes.

The recommendations for countermeasures (either as prevention or 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 Ecological Niche Modeling (ENM) software such as GARP and BIOCLIM. The enhancements on the software and data processing accuracy would improve the predictive potential of these tools. Furthermore, 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 (e.g. Polymerase Chain Reaction) for NIS 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 if 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.

In the final analysis, the data supports that NIS could be used as a BW agent. Vigilance is now required, both nationally and internationally, as the NIS BW attack could arise in any nation or niche at any time. With the time lag before detection of an NIS BW attack being significant, and the abundance of targets available in the global society, the probability is high that rouge nations, criminal organizations, or terrorists (even the "lone wolf" individual) –as opposed to nation states- would strive to use NIS BW.

It is imperative that various national and international organizations as well as intelligence and law enforcement agencies remain on guard to the application of NIS BW in this century.

2. CITATIONS

1- Couch, Dick, Captain, USNR (retired) (2003). The U.S. Armed Forces Nuclear, Biological and Chemical Survival Manual. New York, NY: Basic Books.

2- McKenzie, Kenneth F. "The Revenge of the Melians: Asymmetric Threats and the Next QDR: McNair Paper 62." *Air War College, MAXWELL AIR FORCE BASE, MONTGOMERY, ALABAMA* . INSTITUTE FOR NATIONAL STRATEGIC STUDIES, NATIONAL DEFENSE UNIVERSITY, 2000. Web. 28 Aug 2011.
<http://www.au.af.mil/au/awc/awcgate/ndu/mcnair62.pdf>.