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Analysis of Introduced Species as a Form of Biological Weapon: Part 1-Theory and Approaches

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Abstract

The hypothesis of this paper is that introduced species (aka non-indigenous species) could be used as a form of biological weapon (BW). The first step of this paper is a brief review of biological weapon concepts, and a review of the definition of an introduced species, as well as a brief survey of historical examples of introduced species. Previous evidence of the use of invasive species as a form of biological weapon is also discussed.

The methods to predict a successful invasive species candidate is discussed, and examples of various theories and computer software models to analyze introduced species invasions are reviewed. 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.

This research discusses the approaches of invasive species by hostile actors (aggressor nation, crime syndicate, bioterrorists, or lone individual), as well as the various BW targets (public health, ecosystems, agricultural commodities, biofuel feed stocks). 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 include poor communication between local population and government scientists and decision makers; monoculture of agricultural fields; disturbed or damaged ecosystems, and presence of favored niches in the targeted areas. The strategies to introduce BW invasive species are discussed, and range from human smuggling and delivery by vectors to 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.

Two models are presented to describe the process of invasive species BW by hostile actors-one, using a single invasive species and one leading to invasion meltdown of the targeted area. Four examples of potential BW using introduced species are discussed, with supportive evidence for their effectiveness and invasive potential on targets (Nipah virus, *Striga* plant parasite, Heartwater-*Ehrlichia ruminantium*, and Wheat Stem Rust-*Puccinia graminis f. st. tritici*). Finally, the data supports the hypothesis that introduced species could be used as a form of biological weapon.

Keywords: Agrobioterrorism; Biodefense; Biological weapon; Biosecurity; Bioterrorism; Exotic species; Garp; Heartwater; Introduced species; Invasive species; Nipah; Striga; Wheat stem rust

Introduction

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" [1,2]. Daly [3] states that 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. 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) [4-8]. 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].

Finally, BW can be directed against agricultural targets using various bacterial, viral, and fungal agents (e.g. Anthrax, Foot-And Mouth Disease, Wheat Smut) [11,12]. The reasoning for targeting agriculture, for example, 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 [11]. Therefore, attacking the agricultural

sector could weaken a nation, both internally as well as economically in the global market place.

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 [13,14]. Since the introduced species usually is devoid of the 3 P's (i.e. Predators, Parasites, and Pathogens) to the organism, the population of the nonnative 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 [15] will be used for all introduced species-Non-Indigenous Species (NIS).

Examples of NIS introductions include the accidental introduction

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of the algae, Caulerpa taxifolia, which has been a menace to the Mediterranean coastal ecosystem off of France, Spain and Italy [16]. The deliberate introduction into the U.S. of the black necked pheasants by Judge Owen Nickerson Denny from China; seemingly for hunting purposes [17]. 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 as it spreads [18]. Some NIS organisms have hybridized with other organisms, while others have outcompeted 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))[1,5,6].

Two key points to (*Aedes albopictus*), (*Xanthomonas axonopodis*), Protozoal-(*Plasmodium relictum*), (*Cryphonectria parasitica*), -(*Pueraria lobata*) (*L* note about NIS invasions is the time delay from the entry until establishment of the organism in the host or ecosystem. This time delay may be short or very long (e.g. months or decades), depending on the NIS organism, niche characteristics and number of NIS invasions occurring [14,16-18]. Second, 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) [14,16-18].

Evidence of Prior Nis Use as a BW

To support the hypothesis that Non-Indigenous Species (NIS) can be used as a Biological Weapon (BW), the question arises as to how a deliberate release could be distinguished from an accidental release of NIS into a niche. Although much more research would be needed, at the present time, the following sections of this paper 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 [14,18-21].

One possible prior example of a NIS BW attack in the past is the case of "The Breeders". According to Root-Bernstein [22], 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 [23-25]. 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 [23-25]. During the 1980's, traps were used to monitor for the presence of the Medflies, with subsequent evening spraying of the organophosphate pesticide, Malathion, where traps caught Medflies [26]. 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

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state of California [26]. During this time period, unusual appearances of Medflies appeared in traps in areas previously sprayed and believed to be Medfly free [26]. 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" [22,26,27].

NIS Biological Weapon Determination: Methods to Determine Nis Weaponization and Efficiency

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 section 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 [28], 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 [28].

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 for a society, aside of the aesthetic or biophilic value of the niche [29].

The key questions for weaponization of NIS are: what is the organism and where can it be applied? One limitation to 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, 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 via the NIS BW attacks).

History

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 [18]. In a study by Kolar and Lodge [30], 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 [30]. A study by the National Research Council [14] found that history of an NIS can be a strong indicator of invisibility, and hence, it can be 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 [31]. Ruiz and Carlton [31] 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 due to limited resources to detect high-impact invasions, or the precise timing of invasions; hence, a historical record is limited for many species [31].

The advantages of an NIS history approach to NIS invasiveness 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 previous 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 [31]. These agencies and scientists may have to obtain the limited resources (e.g. funding, manpower, public support, education, communication), to implement counter strategies 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 various NIS species [32,33]. Although skilled bioterrorists and determined agents from rouge states can out maneuver these policies and practices (e.g. smuggling). Furthermore, skilled border agents will have been trained in the detection and interception 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.

One step approach

Beyond the history of any potential NIS species, Peterson et al. [34] 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. 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. [34] describes the one-step approach developed by Hollander et al. [35]. Hollander et al. [35] 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. [34] states that one-step (i.e. onestep, 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 [34]. But, Peterson [34,36] 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 [34].

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.

Two step model

Peterson et al. [34,37] 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 [36]. 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 [36,37]. 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 [36]. 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 [36]. Ecological niches provide a set of possible factors under which a species is able to invade and succeed in a naïve niche [37].

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 [34,37]. 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, as well as the subjectivity of mapping, which includes unsampled areas within maps [34]. 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 data (e.g. decades or older) [34]. 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 [34,37,38].

With the means to obtain primary point occurrence data for a particular species, the development of two-step models can progress [39]. Although other models exist, this paper will focus on two promising models, BIOCLIM and GARP.

Environmental niche model-bioclim

BIOCLIM, (short for BIOCLIMATIC), 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.) [40]. Peterson et al. [34] 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 [40], 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 [41] 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 [41]. Earlier versions of BIOCLIM consisted of 35 climatic parameters throughout the species' known range.

Beaumont et al. [42] 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 [42]. The researchers suggest that BIOCLIM [43] 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 [39] 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 [39].

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 [36]. 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 [43].

Environmental niche Model-Garp

GARP (Genetic Algorithm for Rule-set Prediction) is a genetic algorithm devised by Stockwell [44-46], to address the problem of species distribution modeling. 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 [46]. Stockwell and David [44] notes that a secondary goal was to provide a reasonable explanation for the systems' predictions. 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 [44]. 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 [44]. 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. 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 [34].

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 [44]. 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 [44] (i.e. convergence).

Examples of successful GARP applications include the Greater Glider (*Petauroides volans*)-a gliding possum in forest regions of South eastern Australia [44], the North American invasion of the NIS aquatic plant *Hydrilla verticillana* [36], the Spiny Pocket Mouse (*Heteromys anomalus-Heteromyidae*) in Columbia and Venezuela [47], the Passerine bird (*Carpodacus mexicanus-Fringillidae*) in Western North America and South Mexico [47], the Wood Thrush (*Hylocichla mustelina*) in Maine [34], invasion of the Fire Ant (*Solenopsis invicta*) in North America [48,49], global invasion potential of Witchweed (*Striga*) and Broomrape (*Orobanche*) [50,51], and 34 species of North American passerine birds. Many studies included examining the invasive potential of NIS species. Peterson [36] 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" [36]. 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 [36].

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 [46] 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 [46]. 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) [46]. The consensus approach of multiple models compensates for problems in one model and provides good results on most occasions [52]. Peterson [50] notes that GARP testing has demonstrated insensitivity to dimensionality of environmental data, which is one of the shortcomings of BIOCLIM.

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 [36] 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 [36]. 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 [36].

Another limitation factor for GARP accuracy is the availability of point occurrence data [36]. 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 [45]. Stockwell and David [44] 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) [36,44].

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 "coarse grain" of detail in the map), which leads to more accurate GARP results. Stockwell and Peters [45] 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 (i.e. point occurrence data). Sampling bias can introduce unwanted patterns in the data. Most museum databases records where species were collected, but no information exists on where the species did not occur. This can represent a sampling bias for a particular set of the dependent value (i.e. data of species presence only). Hence, background data, where the species was not present, is absent. GARP provides a solution for this bias 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 actual absent record data is present, it will also be included. This strategy helps to reduce errors in GARP analysis [45].

To counter errors, Peterson and Cohoon [53] 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 [54] demonstrate that in obtaining sample size of species distributions-a law of diminishing return eventually arises. 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 aforementioned 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 [37] 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, in comparing historical data, one step analysis, BIOCLIM, and GARP modeling, GARP modeling would be favored-along with sufficient point of occurrence data of the candidate NIS organism-as a tool for NIS BW development [55-61].

Garp studies with potential bw agents

The concept of using GARP in BW selection and development goes beyond mere speculation [60]. 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 [62] 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. One example is the GARP analysis, which predicted the spatial dynamics of the vector insects and eventual human cases of Dengue Fever in Mexico [63]. 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

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the US BW program in the 1950's [26,64-66], as well as research into using Dengue Fever as a BW agent by the French BW program in the 1960's [67]. Furthermore, Lockwood [26] 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. 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 [68]. 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 [68,69]. Since the disease has a high mortality rate, it was favored for BW research and eventual weaponization by the former Soviet Union [65,70,71], as well as a pathogen of BW research for South Africa [64]. 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 [72,73]. Monkeypox is also listed in the US Military Field Manual of potential biological warfare agents [74].

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 that were sold in pet stores in the Mid-West United States [72,75,76]. Reed et al. [76] 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. [75], 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. [75] and the Reed et al. [76] 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 [77] 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. [72] 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. [72] researched using GARP, what could be the invasive potential of these rats in North

America. 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. [78] 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. [79] 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 [78].

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 [80], 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 [81], with its rapid assessment program [82], along with the Census of Marine Life [83], 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 [84] (a collaboration of Species 2000 and the Integrated Taxonomic Information System), and the Encyclopedia of Life [85]. This data, along with new genetic bar coding performed by the Consortium for the Barcode of Life [86], 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 catalogues 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.) [29,87], this may also indicate invasiveness potential. Some pioneer species have become NIS in naïve environments (e.g. rats) [18]. 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.

Targets and Nis BW Development

Introduction

NIS (Non-Indigenous Species), used as BW (Biological Weapon), can be applied on a variety of targets. The targets could include food

crops for humans or livestock feed [88]. 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. 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) [54].

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 [89-96].

Horn and Breeze [21] 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 [21]. Agricultural exports alone account for \$140 billion and for 860,000 jobs. The United States has been known to have one of the safest, secure and reliable supplies 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 [21].

Yet, as Brown [89] points out, 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.

Parker [90] 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 [90] 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. 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 [90].

The reasons for a BW attack on agriculture can be summarized by Chalk [88], 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 imports suspensions (imposed by such nations as Canada, United States, Denmark, Germany, and Hong Kong) cost Chile over US\$200 million in lost earnings [88].

Another possible outcome from a BW attack on agriculture would be the loss of political support and confidence in the government. Chalk [88] 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 [88]. 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 [88].

The third 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 [88] 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 [88] 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. 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 [88] 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 [88].

Chalk [88] 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 [91] discusses the use of BW against "soft targets", as a form of Fabian strategy of indirect warfare. In essence, Hickson [91] 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 [91]. 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.

Factors to Consider in the Attack and Selection of Targets

Process of attack (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 (Chart 1).

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 [90].

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 whether the target has been 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, and therefore, signal the need for counter strategies, 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; 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 a related species to the NIS candidate organism (by family or by genus), exhibited prior invasiveness traits; and ease of transport, storage and delivery of the NIS propagules. Furthermore,

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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 [19] 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 [19]. For example, Baskin [18] 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 [18].

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 previously, 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 [92] 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 [92].

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 organism and the 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.

BW using insects and other organisms requires knowledge of the mass cultivation of the organisms. For example, Lockwood [27] 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 [27,92,93-96]. 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 et al. and Gelman et al. [97,98].

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 variations in this factor are 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 [18]. 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 [18,99-101]. Any of these delivery routes might be able to bypass Biosecurity protocols with the proper planning. Baskin [18] 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 recipients or knowledgeable accomplices.

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 periods for the NIS BW to be deposited effectively in the target zone? Mack [99] briefly mentions that migrating species have played a role in the distribution of plant species across the globe. Essl et al. [102] 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 [103] 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 [103]. 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 [104]. This is a form of endozoochory (i.e. seed dispersal via ingestion and fecal dispersion by animals).

Finally, dispersal could occur by more technical and precise methods. For example, a cruise missile (aka Biocruise) [105,106], 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 [107].

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 risk of 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, and such technology 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 ability 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 [108,109]. The aggressor may simply wait until the effects of the NIS BW invasion or colonization because the outcomes intended (e.g. economic market shifts, social panic, disease outbreaks, niche collapse, etc.).

Process of Attack-Invasion Meltdown

(Chart- 2: Multiple nis species in bw attack to achieve invasion meltdown)

Although by comparing Chart 1 with 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, multiple NIS species contribute synergistically to accelerate the impact on the naïve ecosystem [52] (Chart 2).

The following steps are similar and differences are noted where necessary:

- Target determination and mission objectives
- Step 1-Biological and Ecological Data of the Target
- **Step 2**-Selections of organisms–NOTE: plural as it is here that the 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 *via* ENM) 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.
- 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.
- 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.

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 [110] paraphrase a threat determination formula devised by Lt. Col. Don Noah, USAF; that is, 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 [93] 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) [93].

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 [32,110-112], 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 [14]. Furthermore, Oppel et al. [113] studied the challenges to eradication of NIS mammals

in islands occupied by humans and domestic animals. Oppel et al. [113] 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 [114]. 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 [18] describes the invasions over four centuries by ships and travelers to Hawaii, and the impact to native species and niche destruction that has occurred.

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. Goka [115] described how the 2004 Japanese "Invasive Alien Species Act" was enacted to control NIS invasions, but a loophole existed that does not address alien microorganisms. Using the NIS invasion of the amphibian chytridiomycosis (*Batrachochytrium dendrobatidis*), as an example, Goka [115] 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.

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 formseconomic, ecological and public health.

Niche Risk Factors

Regarding the importation of non-native organisms, a risk assessment formula and the means to estimate out the NIS risk threat of an organism have been developed [116,117]. Furthermore, the risk of a niche to a particular NIS has been further understood using Environmental Niche Modeling (ENM). 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

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diversity) [102]. 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 [90,93,95,102]. NIS invasions favor low genetic diversity [14,18]. 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 [118] 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 [119]. Kolar and Lodge [30] assert that NIS is 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 [119] 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 [28] 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 [28]. The studies indicate that NIS modification can restructure the ecosystems by modifying disturbance regimes, or adding new disturbances to the ecosystems [28]. With [120] 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) [120]. 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 [119].

Although more research would be needed and welcomed in these topics, the present data offers interesting insights into NIS BW applications and niche vulnerabilities. For example, 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.

Four Examples

It is worth examining several examples of possible NIS BW attacks based on data from various sources. Although more researchespecially 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.

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 [121,122]. Although the reservoir host is fruit bats of the genus Pteropus, the virus can infect pigs, both domestic and feral, as well as humans [121,122]. Evidence exists that not only pig to human transmission occurs, but human to human, as well as pig to other wildlife or domestic animals (eg. cats, dogs, etc.) [122,123]. Weingartl et al. [124] 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 [124]. The Nipah virus can persist over a long period in the patient before causing fatal disease [124]. Nipah will infect both the respiratory and neurological systems [125]. 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 [122]. 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 [126].

The wild pig (Sus Scrofa) was first introduced to the United States from Europe in the 1500's, originally as escaped domesticated pigs [127]. 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]. These organisms are known NIS in US ecosystems. The wild pigs and their hybrid offspring have become a problematic NIS now spanning 39 states in the US [127], including New Hampshire, California, Texas, Hawaii and Florida [128,129]. 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 [127,128,130]. Only two generations are necessary for escaped domestic pigs to revert to feral pigs [131]. Wild feral pigs also can carry a variety of diseases, including Pseudorabies, Swine Brucellosis, Toxoplasma gondii and Trichinella. These diseases can be transmitted to other domesticate livestock, humans and other wildlife [127-129].

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.

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 [132,133]. Striga species are native to Africa, although some species are native to the Indian subcontinent and Australia [132]. 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 [134]. 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) [132,134]. GARP analysis of the NIS global invasive potential of Striga, including for the United States and Mexico has been done [51,135]. Striga invasion into the US Corn Belt would threaten the corn crop valued at \$20 billion annually [135]. 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 [136]. Using artificial stimulants (strigol) and selective herbicides, the witchweed invasion has been stopped in eastern North Carolina fields [136].

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.

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 [92], 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 [92] 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) [54].

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 [137]. 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 [138,139]. 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*) [139]. Cattle egrets have been found to disseminate the ticks among the Caribbean islands, as well as ground dwelling birds [139]. 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 [139]. Furthermore, one recent report of a cattle egret with TBT was found to have migrated to the Florida Keys from the island of Guadeloupe [140].

TBT is a major vector of the rickettsial disease, Heartwater (Ehrlichia ruminantium-formerly Cowdria ruminantium) [141]. Heartwater is an important cause of death for cattle, sheep and goats in Africa [142]. Heartwater has been found to infect rodents, reptiles, birds, lagomorphs and certain carnivores. Heartwater rickettsial organisms infect endothelial and white blood cells [142]. Postmortem of cattle demonstrate edema in the CNS and in the pulmonary region; hence, the name "Heart water" [141]. Furthermore, Burridge et al. [140] 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 [142]. Furthermore, Uilenberg [142] 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 [142]. 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 necessarily indicate absence of Heartwater infection [140]. Also, vertebrates other than mammals can be carriers of Heartwater, including tortoises and indirectly egrets and other migratory birds laden with E. ruminantium infected TBT [142,143]. E. ruminantium can persist in ticks for up to 15 months [144]. Burridge [143] warned of the threat looming to US livestock and deer population from Heartwater. Finally, Allsopp et al. [145] 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 midwestern 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 [146,147]. 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.

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 [102]. Despite eradication efforts, various birds can consume the common barberry fruits and disperse the seeds as they migrate or travel locally [102,104]. 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 [104]. The Barberry is the alternative host for the Wheat Stem Rust fungi. As Schumann [103] 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 [102].

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 [102]. These uredial pustules produce uredospores that provide a "repeating stage" of infection for the wheat plants, and results in wheat field epidemics [102]. These uredospores can spread great distances via winds and transported northward by higher air layers [148]. Later in the season, the dikaryotic mycelium in the wheat stems creates thick walled teliospores that survive the harsh cold winters [102]. The teliospores produce basiodiospores in the spring to start the cycle over again [102]. 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 [102]. Hence, the rust epidemic could die out. Klinkowski [148] notes that Wheat Stem Rust can overwinter on winter wheat in southern Texas. A paper by Madden and Van den Bosch [149] 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 that would provide the between season survival, and reduce the risk of NIS extinction to zero [149].

It must be noted that wheat stem rust was previously developed as a BW agent. For example, Whitby [93] 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. [147] 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 barber, 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 hardly 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 targets of 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 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 crops 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 [150]. 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 [151]. 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. Finally, 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.

Conclusion

The purpose of this paper was to explore supportive evidence for the hypothesis that invasive species (NIS) could be used as a biological weapon (BW). Biological Weapons are defined as "the intentional use by the enemy, of live agent or toxins to cause death and disease among citizens, animals, and plants" [1,2]. 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. Invasive species (aka noninvasive species-NIS) fit most of these criteria, as they are a non-native species introduced into a foreign ecosystem that successfully flourishes and may damage the abiotic or biotic factors of that ecosystem [13,14]. Previous historical events of the Breeders, suggest that NIS BW was previously used by ecoterrorists.

Yet, this hypothesis is further examined using data on NIS traits and history, as well as environmental niche modeling (ENM), as a tool to make reliable predictions of NIS species invasiveness, as well as potential niche impact (i.e. target selection).

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. While no one system was without limitations or disadvantages, the evolution of invasiveness capability determination can be demonstrated in the analysis of NIS history, the one step model originated by Hollander et al. [35], and the environmental niche two step models that connects geographic mapping to the species biology

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 [31]. 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 one step model developed by Hollander et al. [35] was based on mere geographical distribution information based on known point of occurrence data of the species. 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 steps model develops the species model niche in ecological space first, and then projects that model onto geographic space [36]. After the two step model is completed, the model can then be used to predict the range of naïve niches susceptible to NIS invasion [36,37]. 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 [34]. 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 [34]. Although other two step modeling programs exist, this paper focused on two promising models, BIOCLIM and GARP.

BIOCLIM, first used by Nix [40], 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.) [40]. 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 [36,39,41,50]. 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 [36].

GARP is a genetic algorithm devised by Stockwell [44-46]. 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 [34,44,57]. 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 [44]. GARP studies have been successful in a variety of species mapping studies-e.g. vertebrates and invertebrates [36]. 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 [36,46]. Nevertheless, GARP has been a strong predictive modeling tool for NIS invasions [36,49,51,56], as well as for potential BW agents, such as Marburg virus or the vectors of Dengue Fever and Monkeypox [62,63,68,69,72].

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 [82,83], new species from all taxa are still 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.

Furthermore, 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 or not to undertake a NIS BW attack. Two suggested models of an NIS BW attack are presented, which can be 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 strategies to introduce BW invasive species are discussed and range from human smuggling and delivery by vectors to 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 [18,105,106]. Other factors to be considered are 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.

To further support the NIS BW hypothesis, four possible examples of NIS BW and their potential targets are analyzed (Nipah virus-humans, domestic animals, and wildlife; *Striga* plant parasite-corn crops, foodstuffs and corn based biofuels; Heartwater-*Ehrlichia ruminantium*-wildlife, ecosystems, farm animals and potentially humans; and Wheat Stem Rust-*Puccinia graminis f. st. tritici*-wheat crops and wheat commodity markets). 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 the NIS candidate 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.

Although more data and research would be needed, this paper provides a framework (supported by the available data), for how NIS could be used as a BW, and how these NIS BW attacks could be applied. Hence, the present data and format support the hypothesis that nonindigenous species (NIS) could be used as a biological weapon (BW).

This research is significant, as it provides data to support a threat to biosafety and biosecurity by using NIS in BW attacks to various targets (public health, ecosystems, agricultural commodities, biofuel feed stocks). Further research and discussion is warranted to explore rapid detection and eradication countermeasures from an NIS BW attack

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