

The Growing Threat of Agroterrorism and Strategies for Agricultural Defense

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Abstract

Due to the dynamic nature of human conflict, non-traditional terror tactics have evolved to undermine the socioeconomic stability of targeted societies. Considering the landscape in which terrorists operate, emphasis on more subversive methods of biological terror have become prominent in recent decades. Agroterrorism, or the use of plant pathogens to infect a nation's cultivated crops, is an emerging topic due to its threat to global food security and economic stability. Although emergency preparedness objectives have been enacted at national, state, and even local levels, preemptive measures can no longer remain the sole responsibility of intelligence and law enforcement agencies. The agricultural and scientific communities are responsible for collaboration to improve security and pioneer new methods of disease resistance in susceptible crops. Plant immunology is an expanding field which explores the molecular defense mechanisms innately present within the plant kingdom and provides insight concerning novel methods of boosting the immunity of susceptible crops to existing and emerging pathogenic agents. This thesis serves to define the threat of agroterrorism from a national security and scientific perspective, identify notable plant pathogens, provide a brief survey of plant immunology, and discuss topics which can aid scientists, policymakers, and growers in efforts to secure the global food supply from those who would cause harm.

Keywords: terrorism, agroterrorism, biosecurity, agriculture, plant immunology

The Growing Threat of Agroterrorism and Strategies for Agricultural Defense

Following the attacks of September 11 and the anthrax scare of late 2001, non-state actors with the ability to wield unconventional weapons emerged as a primary force within the realm of international security. The use of biological agents to induce public panic is an ancient tactic which garnered particular attention during the post 9/11 era in accordance with rapid advancements in biotechnology. While the threat of bioterrorism has been recognized and addressed by public health and law enforcement agencies, the majority of research and emergency preparedness centers on the immediate threat posed by direct infection of human targets rather than the various indirect methods of bioviolence available to radical groups.

Crop production provides a particularly susceptible target for bioterrorism, as both world powers and developing nations depend upon agricultural development for revenue and sustainment. Numerous scenarios exist detailing the socioeconomic consequences of a direct biological attack, yet threat assessment for the agricultural industry has often failed to reflect the degree of risk. Heightened awareness of the potential for agroterrorism is vital for the implementation of prevention measures, as such success depends upon the practices of individual growers, companies, and scientists. The goal of this thesis is to promote a greater understanding of the indirect threats posed by a lesser-publicized form of bioterrorism and provide suggestions for preventative measures which may be taken by both the agricultural and scientific communities to defend a nation's cultivated crops against the targeted use of plant pathogens.

Defining Agroterrorism

Agroterrorism is the deliberate introduction of a toxic or pathogenic agent into livestock, crops, or the water supply in order to cause disease, mortality, economic loss, and social

upheaval (Gill, 2015 and Green et al., 2017). Such attacks typically grow out of unresolved social, political, economic, or religious contradictions (Alekseeva et al., 2017) and remain the fascination of state and non-state actors (Jaspal and Khan, 2017). The use of bacterial, viral, fungal, or toxic agents to infect plants at any stage of the food production or distribution process has the potential to devastatingly impact one of a nation's most valuable economic and infrastructural resources – its agricultural sector. Agroterrorism has been identified as an increasing threat due to the implications of halting agricultural development, which accounts for approximately one-third of global gross domestic product (World Bank, 2018). Examples in recent history include a forty percent decrease in Israeli citrus exports during the 1970s due to contamination of Jaffa oranges with mercury, as well as a 200-million dollar trade loss in 1989 due to cyanide contamination of grapes in Chile (Alekseeva et al., 2017). It is projected that an agroterror attack on livestock alone could cause 10-30 billion USD in damage to the economy (Alekseeva et al., 2017).

The diversity of agroterrorism threats poses a challenge to food defense efforts for multiple reasons. First, plant pathogens can range from bacteria, viruses, fungi or protozoa to insects or non-native plant species (Green et al., 2017). Pathogenic and parasitic organisms are naturally responsible for 20–40% yield loss globally (Das et al., 2019); thus, the impact of deliberate crop infection would result in devastating consequences for consumers. Secondly, plant, animal, or human mortality may be the objective, or an attack may be implemented with the additional purpose of undermining social stability, holding a nation economically hostage, and causing general loss of confidence in the government (Olson, 2012). Human morbidity and mortality has traditionally been attributed to the dissemination of toxins or bacterial pathogens

such as *Clostridium botulinum*, *Escherichia coli*, and *Salmonella enterica* via the food production and distribution chain (Olson, 2012). However, longer-lasting economic crises may be generated by the infection of plants or animals with disease-causing agents in order to drastically decrease availability of food resources either for domestic consumption or export. Agroterrorism is not only a threat to primary production (upstream) elements of the food chain such as farming, but may occur through imported foods which may be contaminated with pathogens or insect vectors prior to arriving at a port of entry (Gill, 2015). This particular type of bioterrorism has been labeled as “low tech, but high impact” (Green et al., 2017, p. 33) with a high potential for effectiveness due to its low risk for the perpetrator, lack of recognition by agricultural and scientific communities worldwide, and resemblance to natural outbreaks.

Threat Assessment

The threat level of agroterrorism is related to the goal of active terrorist cells on the international scene. Organizations which hope to destabilize nations for political or religious gain may consider strategies involving agroterrorism and should warrant monitoring via cooperative effort between the scientific, agricultural, and intelligence communities. In the past, agroterrorism has not elicited the same level of concern as suicide bombings and mass shootings. This is due to the fact that these methods of attack pose a more imminent threat to the immediate safety of citizens and have been the traditionally preferred tactics of terrorist organizations for decades. However, Gill (2015) notes that the use of bioterror agents must not be overlooked, as this approach is more practical than the use of explosives. Biological agents are often naturally-occurring in the environment or require only a limited microbiological background in order to culture in a laboratory; additionally, these organisms are not as readily detectable by technology

as other weapons of terror (Gill, 2015). Minimal infective doses result in ease of concealment for certain pathogenic agents, and the incubation time of many viruses enables terrorists to vacate the scene to avoid implication or infection (Alekseeva et al., 2017). Another benefit to agroterrorists is the lack of detection methods for pathogenic microorganisms before symptoms show (Alekseeva et al., 2017). Zamir (2016) notes that phytosanitary certificates are often granted based on visual evaluation of plant health; this becomes a major concern when dealing with fungi which produce spores which may be carried by plants but do not necessarily cause disease symptoms. Another problem revolves around the assumption that target hosts will be taxonomically related to plants affected in the country of origin, which does not account for the fact that hitchhiking pathogens may find a much more tolerable environment in the place they end up, resulting in an unexpected host range (Zamir, 2016). As international commerce in mail-ordered seeds and plants grows (Zamir, 2016), this becomes an increasing concern. Thus, agroterrorism is dangerous and damaging in its simplicity, providing a less expensive and less technical method of maximizing impact radius and longevity.

Concern over the use of anti-crop weapons by certain aggressor nations arose in the late 1980s over evidence that Iraq was acquiring the military capacity to destroy Iranian crops via research and development of *Tilletia caries*, *T. tritici* and aflatoxin-producing strains of the fungus *Aspergillus* (Suffert et al., 2009). Islamic terrorist organizations al-Qaeda and the Taliban are known to have contemplated the use of agroterrorism through the weaponization of wheat rust (Suffert et al., 2009), documenting the potential impact of an agricultural terror attack on the United States economy (Gill, 2015). Olson notes that this is consistent with the “death by a thousand cuts” strategy (2012, para. 10), which seeks to “exhaust, overwhelm, and distract U.S.

Department of Homeland Security forces... by flooding America's already information-overloaded intelligence systems with myriad threats and background noise" (2012, para. 10).

Agroterrorism may thus be used independently or as a form of enhancement when coordinated with other attacks, which could include cyberterrorism or smaller, individual bombings (Olson, 2012). According to the Federation of American Scientists (FAS), plant pathogens could be introduced through the food supply chain at various vulnerable entry points (2011). Several risk factors which have been identified include geographical and operational concentration, long-term and insecure storage, reduced isolation, reliance on artificial pest control, and lack of genetic diversity (Green et al., 2017). Historically, the global threat of agroterrorism was limited by poor transportation and technical skill; however, the development of agroterrorism as a viable threat has coincided with the expansion and general availability of scientific knowledge and techniques (Alekseeva et al., 2017). A potential force multiplier is the cultivation of genetically modified strains for which vaccines or treatment therapies do not yet exist. Terrorists with adequate funding and biological knowledge have the ability to engineer resistant strains of plant viruses, enhance toxicity or virulence factors, or acquire experimental species to achieve maximum damage in the event of an attack. Because the majority of risk assessments are based on organisms already known to cause damage, novel aggressive pathogens may not be properly identified by the international community – despite the fact that ninety percent of the threat is linked to such unidentified agents (Zamir, 2016).

Survey of Plant Pathogens

High on the watch-list for plant pathogens are fungal diseases such as wheat smut, rice blast, brown stripe mildew (corn), and karnal bunt (wheat); fungal spores are also a concern, as

they are naturally disseminated through the air and undergo an incubation period during which they are relatively undetectable by workers on the ground (FAS, 2011). Certain bioaerosols such as rust spores can be transported kilometers into the atmosphere, across continents, and may even be spread by air streamlines around falling raindrops (Kim et al., 2019). Because of this natural propensity to spread due to simple fluctuations in weather phenomena such as rain or wind, fungal agents have been effectively used as infectious agents in the past. As mentioned previously, Saddam Hussein used cannisters to disperse the wheat smut pathogen over Iranian fields during the Iran-Iraq War during the 1980s (FAS, 2011). Additionally, fungal toxins – including those that cause damage to plants directly and those which contaminate harvested foods and impact the health of consumers – are a significant threat to the public as well as the agriculture industry. One particular example is aflatoxin, a known carcinogen which binds DNA and proteins (Anderson, 2012). Aflatoxins, produced by the fungi *Aspergillus flavus* and *parasiticus*, could serve as a particularly panic-inducing bioweapon, due to long-term consequences to public health. Plant virology is another particularly impactful discipline on the study of agroterrorism, as genetic advancements continue to be made that grant potential agroterrorists the opportunity to develop new viral strains with no readily available therapy. Plant virus infectious clones are a potential threat due to their inherent pathogenicity and the impact of introduced genetic modifications (Brewer et al., 2018). Furthermore, bacterial plant pathology identifies organisms such as *Ralstonia solanacearum*, *Agrobacterium tumefaciens*, *Xanthomonas oryzae* pv. *oryzae*, and *Xylella fastidiosa* as top threats to staple crops. For each of these biological agents, consideration must be given to virulence, length of incubation period, available therapies, and pathogen resistance factors. The Risk Evaluation Scheme (RES) devised

by Suffert et al. (2009) includes thirty-five fungal, nine bacterial, and six viral agents as candidates for agroterror activities and notes that thirty-two out of the fifty would cause direct crop loss to staple food crops, forest trees, industrial and market crops, and orchards.

Economic and Social Impact

It is vital to note the expected economic and social impact of a potential agroterror attack in order to develop thorough and efficient preparedness strategies. Historically, plant disease has precipitated ecological and social damage as evidenced by high mortality and migration rates (Velásquez et al., 2018). Perhaps the most infamous case was the Irish potato famine of the 1840s, caused by the fungus-like organism *Phytophthora infestans*, in which over a million individuals died of starvation or disease resulting from the infection of Ireland's primary food source (DoChara, 2008). While modern societies are not reliant on monoculture, measures must still be taken to enhance the resistance of food crops essential for human nutrition. With an increasingly global society, national economies are intimately connected to the world market. Gill (2015) specifies that agroterrorism is not "flashy" (p. 10); while it may not produce the shock value of a bombing or other tactic, its use as economic sabotage will result in fear, instability, and loss of life and investments, including "heritage and environmental loss which may have psychological effects on populations" (Suffert et al., 2017, 225). Statistically, 1 in 6 jobs in the United States is linked to agriculture (Olson, 2012). Thus, it is vital to remember that an attack would not only inflict economic hardship on individuals working within the agriculture industry, but would inevitably spiral outward to impact the rest of the nation; the severity of the effects would depend on the type of pathogen.

In the recent past, analysts expressed concern that groups such as al-Qaeda would adopt tactics utilizing agroterrorism due to a “highly favorable cost-benefit ratio” (FAS, 2011, Section 6, para. 2); however, more recently the concern has shifted to include economic opportunists, domestic terrorists (including lone wolves and disgruntled employees), as well as militant animal rights groups (Gill, 2015). The Federal Bureau of Investigation (FBI) notes the extent of economic and social damage which an agroterrorism attack could inflict, particularly on the United States:

[These include] direct losses due to containment measures, such as stop-movement orders (SMOs)... [and] indirect multiplier effects, such as compensation to farmers for destruction of agricultural commodities and losses suffered by directly and indirectly related industries... International costs would result from protective trade embargoes. Less measurable consequences would include the undermining of confidence in and support of government, creation of social panic, and threat to public health on the national and global levels. (Olson, 2012, para. 14)

An example of the economic devastation which can result from even a benign food issue can be found in the Korean beef riots of summer 2008. Rallies against U.S. beef imports resulted in demonstrations involving thousands of citizens and is credited with undermining the recently elected administration of South Korea (Green et al., 2017). The likelihood of food shortage resulting from disruption of the food supply is compounded in urban areas, as supermarkets only stock a week’s supply of food with the expectation for arrival of a timely shipment (Olson, 2012). The panic and rioting which would occur as a result of a successful agroterror attack and subsequent food shortage cannot be overstated. In the event of a large-scale attack, the resulting

economic and social upheaval has been projected to cause extensive, long-term damage with more persistent consequences than most traditional tactics, such as suicide bombing.

Consequently, agricultural losses are considered a primary obstacle to global food security for a growing population (Velásquez et al., 2018). Plant disease poses a threat due to the longevity of infection; whereas a biological toxin or chemical contaminant may be isolated quickly and the affected food products destroyed, infection at the source impacts growth and harvesting of cultivated crops fundamental to food security.

Prevention and Preparedness Measures

Olson (2012) notes the importance of involving law enforcement agencies in the counter-agroterrorism objective, as warning signs such as theft of vaccines, medicines, and agricultural equipment may be preliminary warning signs of an agroterror attack. Integral to threat-mitigation is cooperation not only amongst law enforcement and intelligence agencies, but also between the agricultural and scientific communities. This includes involvement and proper incident reporting by farm workers and first responders such as private and state veterinarians, phytologists, police, extension agents, and local authority contingency planners (Green et al., 2017). A major tenet of the preemptive legislation implemented by the United States government hinges on the cooperation of multiple agencies including the Department of Health, Department of Agriculture (USDA), Federal Bureau of Investigation (FBI), and the Department of Health and Human Services (Hunter 2015). The challenge for agencies such as the Food and Drug Administration (FDA) has been the shift of perspective from food safety to food defense (Gill, 2015); while the former is primarily focused on preventing contamination, the latter focuses on preemptive strategies for protecting the ingredients of human food or animal feed. Furthermore, the

importance of differentiating between accidental and deliberate attacks must be understood; scenario-based training exercises have been effective in the United States to streamline the process of engaging both the USDA (for containment and restoration) and law enforcement (for investigation and evidence gathering) in the event of an attack. Classical epidemiological methodology as well as the application of forensic science, in which scientific knowledge and technology provide evidence in possible violations of law, is essential (Suffert et al., 2009). Research facilities have been identified as potential points of entry, and the threat includes failure to maintain security at such locations due to mild disagreements between advocates for freedom of research and national security officials (Suffert et al., 2009). Recommendations have included use of molecular-based detection strategies for early detection and identification of plant pathogens in order to flag the emergence of epidemics deemed suspicious and the creation of nationwide diagnostic networks (Suffert et al., 2009). One counter-agroterror tactic adopted by the United Kingdom is the establishment of international trade links to alternative food sources (Green et al., 2017). This strategy is projected to alleviate a food shortage in the event of a crisis and may be integral to the survival of developing nations in the event of an agroterror attack. However, it cannot be relied upon as a primary protective policy for nations such as the United States, which are among the top producers of the world's food crops.

Achievement of crop biosecurity has been attempted by quarantine and phytosanitary regulations enacted by organizations such as the International Plant Protection Convention (IPPC), which strives to harmonize efforts to prevent the introduction of exotic plant pests in various nations (Suffert et al., 2009). The importance of countering illegal transportation of plant products across national borders by the establishment of biosecurity checkpoints has been noted

(Jaspal and Khan, 2017), and as recently as October 2019, the U.S. House of Representatives recommended the Protecting America's Food and Agriculture Act to the Committee on Homeland Security and the Committee on Agriculture. The act states that, "It is in the national security interest of the United States to ensure that the Nation's food supply is sufficiently protected...[through] the availability of adequate resources at the border to conduct inspections of incoming food and agricultural goods" (S. 2107, 2019). An increased number of Customs and Border Protection (CBP) Agriculture Specialists and support staff within the Office of Field Operations is proposed, as is a comprehensive review of the coordinated efforts between associated federal agencies to address risks to the agricultural supply, including inspection of agricultural commodities entering the United States and the training provided to CBP Agriculture Specialists. This type of preventative measure would be complemented by foodborne outbreak emergency exercises such as Norway's Stella Polaris exercise involving local, regional, and national units over a several-day period; this nation-wide training exercise has been credited with high levels of learning concerning general and specific contingency plans (Wahl et al., 2015). The Stella Polaris exercise highlighted the necessity of crisis communication (including media handling) as an integrated element in functional emergency exercises, as findings indicated that personnel felt ill-equipped to handle the media aspect of emergency response (Wahl et al., 2015). Research also suggests that lack of public awareness, particularly within academia, remains a roadblock to food defense efforts. Jaspal and Khan (2017) recommend addressing this issue through increased emphasis on plant biosecurity, rather than simply bioterrorism, within life science curriculums.

Overview of Innate Immunological Defense Mechanisms

While the immune system of vertebrates – particularly animals and humans – has been studied extensively at the molecular level, that of plants has remained an enigma until recent years. With such growing concern surrounding the agriculture industry due to the potential for terroristic threats, research concerning the defense mechanisms that innately exist within plants has been conducted to determine how best to protect valuable food resources. Macho and Zipfel (2014) note that for survival, the plant immune system must have the capacity to respond to biotic or abiotic and internal or external stimuli simultaneously at the cellular, tissue, and organ level. However, due to their sessile nature, plants differ from other living organisms in the manner by which they must defend against pathogenic bacteria, viruses, and fungi. Unlike humans and animals, plants do not have the capability to mount an adaptive immune response to foreign invaders and lack specialized mobile immune cells; instead, plants rely on the innate immune capability of individual cells and systemic signaling from infection sites (Jones and Dangl, 2006).

Two main types of immunity exist within the plant kingdom – constitutive and inducible, with the latter further branching into pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). These responses work simultaneously to provide the most efficient and effective immunity possible to the organism. Various systemic responses may also be engaged in the event of pathogen invasion; these responses are named according to the method of induced immune response. Despite lacking the capability to develop an acquired response, plants do maintain their own form of immunological memory through defense priming – which falls under the inducible response and can be either naturally or artificially induced – as well as heritable

systemic immunity. Thus, when considering how to best protect crops from both natural disease and sabotage, methods of boosting the innate immunity and systemic signaling capabilities of individual organisms must be considered.

Constitutive Immunity

Plants, much like humans, contain a first-line of defense against microbial infection. Similar to the epidermis, most plants maintain a waxy cuticle surrounding their shoot (the above-ground portion of the plant). In perennials, this is known as the periderm and consists of non-living cork cells (Reimer-Michalski and Conrath, 2016). This layer poses the first line of defense for potential pathogens and requires specific environmental and virulence factors to overcome. This constitutive defense mechanism as well as the synthesis and accumulation of phytoalexins, glucosinolates, and other secondary metabolites (Reimer-Michalski and Conrath, 2016) make up what is known as Non-host Resistance (NHR), a pre-invasion immunological defense system that is the most prevalent form of plant immunity. Studies in *Arabidopsis thaliana* have shown that plasma membrane-localized ATP-binding cassette (ABC) transporter PEN3 and myrosinase PEN2 interact in some manner to export antimicrobial secondary metabolites and confer constitutive immunity (Reimer-Michalski and Conrath, 2016). Another aspect of NHR is formation of cell wall appositions via a Soluble NSF Attachment Protein Receptor (SNARE) complex formed by the PEN1 syntaxin which engages in vesicle secretion at the site of fungal invasions (Jones and Dangl, 2006).

Inducible Immunity

The inducible aspect of plant immunity may be classified as either pattern-triggered Immunity (PTI) or effector-triggered immunity (ETI), depending on the type of response

initiated (Li et al., 2016). PTI (Figure 1A) occurs when pattern-recognition receptors (PRRs) located at the cell surface (Macho and Zipfel, 2014) recognize microbe-associated molecular patterns (MAMPs). These may be either infectious non-self determinants or self-molecules known as damage-associated molecular patterns released upon pathogen perception or induced cell damage (Macho and Zipfel, 2014). Multiprotein complexes at the plasma membrane contain PRRs as well as additional transmembrane and cytosolic kinases necessary for both initiation and specificity of immune signaling, regulated by protein phosphatases and E3 ligases (Macho and Zipfel, 2014). While this is similar to innate immunity in vertebrates, the main differences revolve around the multiplicity of PRRs – compared to the limited number required in animals to recognize more highly conserved PAMPs – as well as the lack of intracellular PRRs found in plants (Reimer-Michalski and Conrath, 2016). However, this response has been shown to be incredibly complex, involving co-receptors, negative regulators, substrates linking PRR activation to the induction of early signaling components, and initiation of signaling via phosphorylation (Macho and Zipfel, 2014). Li et al. (2016) note that a cascade of phosphorylation from MAPK to transcription factors is needed for instantaneous transduction of MAMP signals to transcriptional machinery, and that this process is the major governing factor of the transcriptional selectivity of primary immune genes. PTI is often all that is needed to halt further colonization and is deployed against all types of microorganisms, whether infectious or not (Jones and Dangl, 2006). PTI works through various MAMP/PRR interactions; the most prominent of these include activation of FLAGELLIN-SENSING2 (FLS2), a leucine-rich repeat receptor kinase (LRR-RK), by the flg22 amino acid sequence as well as recognition of bacterial elongation factor Tu and subsequent EF-Tu/EFR pairing (Reimer-Michalski and Conrath, 2016;

Macho and Zipfel, 2014). Fungi also contain MAMPs that result in pairing, such as fungal chitin/CERK1, studied in *Arabidopsis* (Reimer-Michalski and Conrath, 2016). These MAMP/receptor kinase pairings result in downstream cellular defense signaling (Reimer-Michalski and Conrath, 2016) that is known to cause reactive oxygen species (ROS) release, mitogen-activated protein kinase (MAPK) activation, plant hormone synthesis and signaling, metabolic changes, excessive transcriptional reprogramming, and the expression of immune-related genes (Reimer-Michalski and Conrath, 2016; Macho and Zipfel, 2014). In fact, MAMP perception has been demonstrated to induce approximately one thousand genes within an hour after exposure (Li et al., 2016). Macho and Zipfel (2014) conclude that the biological importance of this primary immune defense is emphasized by the necessity of evasion or suppression by an adapted pathogen in order for disease to occur.

In some cases, a pathogenic microorganism possesses certain virulence factors which enable it to avoid recognition and binding by PRRs. These virulence factors are known as effector molecules and are released into the plant cell (Jones and Dangl, 2006). This stimulates ETI (Figure 1C) via direct or indirect recognition by Resistance (R) proteins (Reimer-Michalski and Conrath, 2016) and nucleotide-binding oligomerization domain-like receptors (NLRs) within the cell (Macho and Zipfel, 2014). The guard hypothesis is used to describe this immune function, in which so called “watchdog” R proteins (Reimer-Michalski and Conrath, 2016) monitor cellular protein integrity and note structural changes due to effector molecules and may then initiate the hypersensitive cell death response (HR), a form of programmed cell death. An NB-LRR protein recognizes the particular effector which has invaded the plant cell, resulting in a next-level response stronger and more efficient than PTI and similar to the Apaf-1 induced

proximity mechanism of apoptosis in animal cells (Jones and Dangl, 2006). This occurs only as a means of ridding the plant of the infected cell; it has not been shown to specifically impact pathogen growth in any case other than haustorial parasites (Jones and Dangl, 2006).

Systemic Immunity

A localized infection, if not deterred by inducible defenses, either induces the hypersensitive response or results in disease symptoms (Figure 1B). When this occurs, a different type of immunity is engaged; systemic acquired resistance (SAR) defends against reinfection throughout the entirety of the organism, up to the entire lifespan of the plant. It also is a heritable trait (Reimer-Michalski and Conrath, 2016). Another type of systemic immunity is known as induced systemic resistance (ISR) which is activated specifically by root-colonizing or necrotizing bacteria and fungi (Reimer-Michalski and Conrath, 2016). Wound-induced resistance (WIR), which involves a buildup of protease inhibitors responsible for preventing insect digestion of plant material (amongst other things), can defend against microbes or external pests. Thus, this type of immunity typically occurs during infestation of some kind – either for feeding or egg deposition purposes (Reimer-Michalski and Conrath, 2016) – but is also noted in physical injury to the plant. Other environmental conditions, such as metabolic disturbances, can also induce forms of systemic immunity. Reimer-Michalski and Conrath note that SAR and ISR are the immune responses with the greatest promise for sustainable agriculture due to their broad-spectrum activity and lack of negative effects on overall plant fitness (2016); however, the researchers also note that alterations to primary metabolism in tubers and leaves have aided in the prevention of future infection by organisms such as *Pectobacterium atrosepticum* (soft-rot), *Alternaria solani* (early blight), and *Phytophthora infestans* (late blight). Due to the manner by

which SAR and similar forms of systemic immunity function in plants, their likely applications in agriculture are related to regular fluctuations in environmental and growing conditions. When considering methods for combating agroterrorism, optimizing inducible immunity such as PTI or ETI, or perhaps even preventing invasion of the pathogen completely through artificial boosting of constitutive immunity are better options to explore. Following colonization and the appearance of symptoms, even in the event of recovery and development of systemic immunity to prevent infection of future crops, damage for the current growing season may be inevitable.

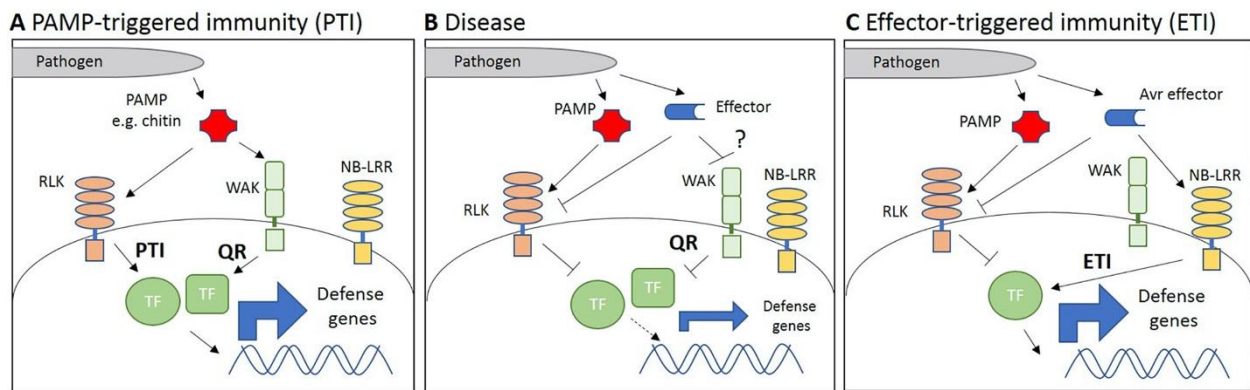


Figure 1. A visual representation of the molecular activity resulting from (A) pattern or PAMP-triggered immunity, (B) infection by a plant pathogen, and (C) effector-triggered immunity (Van de Wouw and Idnurm, 2019).

Defense Priming

The memory aspect of plant immunity does not come in the form of mobile, specialized function cells with the sole purpose of mounting an improved response upon reinfection. Instead, a system of high alert is maintained within both the exposed tissues as well as the uninfected regions in a plant after initial stimulation of the immune system; a good analogy would be a country which spends resources building up defense technology (plants) versus one which maintains a small standing army (vertebrates). Defense priming is defined as the aspect of inducible plant immunity which influences response to reinfection or abiotic stress via

recognition of MAMPs, herbivore-associated molecular patterns, DAMPs, pathogen effectors, or certain xenobiotics (Reimer-Michalski and Conrath, 2016). A memory response is created by the modification of histones (H3K4me3), demethylation of DNA in defense gene promoters, increase of PRRs and coreceptors in the cell membrane, and enhanced levels of dormant MPKs. Defense priming thus provides a similar primed innate response to that of mammals, providing a long-lasting epigenetic memory (Reimer-Michalski and Conrath, 2016).

Reimer-Michalski and Conrath (2016) note that defense priming is particularly effective in perennials and has been shown to have a more positive impact on the inducible immune response than the accumulation of antimicrobial proteins that can be activated in plants by treatment with SA, Pip, (Me)JA, Aza, and certain xenobiotics in stress-free scenarios in such a manner as to only minorly impact plant fitness. A continuing problem identified by researchers studying food security and defense is the reliance on artificial pest control and a lack of genetic diversity in crops (Green et al., 2017). Jones and Dangl (2006) view defense priming as an opportunity to translate knowledge of the plant immune system to practice, utilizing molecular techniques to address such concerns and improve sustainable agriculture and food defense measures; implementation of their suggestions on a wider scale is perhaps one method of combating plant pathogens.

Biotechnology and Sustainable Agriculture

The immune capability of plants may not consist of an acquired response, yet plant immunology is far from a simplistic topic. Looking to the future, further study of the molecular mechanisms behind plant immunity – particularly, transcriptional regulation in pattern-triggered immunity and controlled defense priming – should be considered, with experimental projects

evaluating the validity of artificial induction of innate defenses. Due to the development of an increasingly global society, a novel approach to food defense and biosecurity is needed to protect both world markets and individual consumers of agricultural products. Thus, plant breeding programs should focus on incorporating “abiotic tolerance, growth, and biotic resistance variability that favor plant immunity and disfavor pathogen virulence” (Velásquez et al., 2018, p. R628). Many researchers argue that the scientific community should be at the forefront of biosecurity initiatives, focusing on offensive breeding efforts rather than defensive policing measures. One proposed solution is an internationally coordinated effort to enhance biodiversity (Zamir, 2016) through a network of sites collaborating on the sequencing of plant and pathogen genomes to determine genetic markers and a cloud-based network integrating such genetic information with global distribution. This hybridization project would require partnerships between state-sponsored and private academic institutions already invested in plant genomics, informatics, seed storage, and biodiversity education (Zamir, 2016). Current research centers on boosting host resistance through a variety of methods including targeted genome editing, stimulation of host resistance genes with antiviral activity, activation of autophagy, and synthesis and utilization of bacteriocins.

Resistance Selection

One of the most promising strategies for developing host resistance in market crops is the artificial activation of resistance genes associated with PTI or ETI. While plant genome sequencing to determine the specific genes active during an infection has been successfully performed, this task can be made much simpler by the study of pathogen effector molecules recognized by these genes (Van de Wouw and Idnurm, 2019). Effector molecules have been

utilized to guide breeding of resistance genes and trigger defense responses within plants infected by fungi or oomycetes, and the success of such biotechnology should encourage the use of effector molecules to study diseases across a wider range of plant-pathogen interactions (Van de Wouw and Idnurm, 2019). The use of genetically modified crops in this analysis is an invaluable tool, as transgenic creation of resistance genes rather than the slower traditional method of cross-breeding saves time and enables resistance selection in crops incapable of conventional breeding; it also prevents the reduction in yield and fitness often observed in selection of genetic resistance, a phenomenon known as linkage drag (Van de Wouw and Idnurm, 2019). A recent study by Xu et al. (2017) explored engineered resistance in relation to fitness cost (i.e., the ability to replicate and survive in a competitive environment) by studying *Arabidopsis thaliana* and the *AtNPR1* gene. The researchers determined that tighter control of defense protein translation triggers the *TBFI*-cassette, consisting of the promoter as well as two upstream open reading frames (uORF_{STBFI}) of the *TBFI* gene. *TBFI* is responsible for switching the organism from growth-related activities to defense-related translation, and the cost to benefit ratio of this method remains favorable both in the laboratory and the field (Xu et al., 2017).

A potential problem with targeting resistance (R) genes in plants infected with viruses, bacteria, or fungi is the corresponding development of resistance in pathogen species. Das et al. (2019) note a necessary shift in focus from targeting R genes to editing S genes, also known as host susceptibility factors. Use of the CRISPR-Cas9 gene editing system (Figure 2) has garnered attention in plant genome modification due to its efficiency and cost-effectiveness, which is unattainable by traditional breeding methods. Additionally, this aspect of biotechnology requires only a single guide RNA (sgRNA) to provide target-site specificity; it also allows for multiplex

genome editing or targeting several genes with one construct (Xu et al., 2017). Although the CRISPR-Cas9 system has been utilized in the study of engineered plant resistance, further developments must be made before its broader implementation. Minor errors from off-targeting as well as bioethical concerns have kept CRISPR-Cas9 products from being widely disseminated in the real world (Xu et al., 2017), which is why continued study of the longevity and fitness of organisms with CRISPR-engineered resistance in the field is necessary to improve current agricultural defense mechanisms (Van de Wouw and Idnurm, 2019).

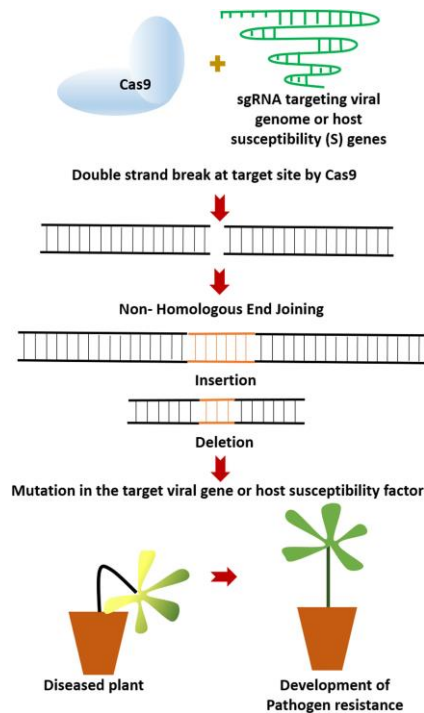


Figure 2. The CRISPR-Cas9 system for genome editing and engineering of plant resistance utilizes a single guide RNA (Das et al., 2019).

Host Antiviral Activity

Resistance to plant viruses may be engineered through gene editing. It has been determined that antiviral defense mechanisms are mediated by host factors which target viral nucleic acids and proteins through autophagy, proteasome degradation, RNA decay and gene

silencing (Garcia-Ruiz, 2018). Antiviral gene silencing recognizes viral RNA through formation of hairpin structures, replication intermediates, and overlapping transcriptional products and targets these single-stranded RNAs for degradation or translational repression (Garcia-Ruiz, 2018), resulting in a plant's recovery from viral symptoms due to the inability of the virus to replicate its genome or mobilize. Another opposite approach involves the identification of host pro-viral factors, or the characterization of genes which make a plant susceptible to pathogenic infection. Genome editing is still necessary utilizing this method; however, rather than targeting the virus, a focus on host pro-viral factors transforms a permissive host into a non-host through inactivation of susceptibility genes (Garcia-Ruiz, 2018). Although this has proven effective in preventing infection and viral replication, certain challenges do exist – particularly, whether or not the pro-viral factor is an essential gene required for host survival. Garcia-Ruiz notes that viruses such as orthospoviruses, potyviruses, tobamoviruses and geminiviruses which cause plant diseases in staple crops such as maize and sugarcane will likely receive more attention in the near future through a combination of genome editing and epitope-tagging of viral proteins (Garcia-Ruiz, 2018). This would streamline the process of determining host genes which display antiviral as well as pro-viral characteristics for the adoption of a synergistic approach to developing viral resistance.

Antimicrobial Peptides

Secondary bacterial metabolites of the *Bacillus* species (Figure 3) have proven to be successful antimicrobial substances. Nonribosomally-synthesized peptides and lipopeptides displaying antagonistic properties against invading species have been utilized as natural alternatives to chemical pesticides and are a simpler defense mechanism than genetic engineering

(Fira et al., 2018). Certain species such as *Bacillus subtilis* or *Bacillus amyloliquefaciens* FZB42 utilize 4-8% of their genome for the synthesis of dozens of such antimicrobial peptides, and other strains produce secondary metabolites which not only impede pathogen growth but also contain growth-promoting properties for the plants with which they associate (Fira et al., 2018). The process of antibiosis occurs when the small, heat stable, amphiphilic proteins that characterize non-ribosomal peptides interact with the envelope of an invading cell, leading to its demise by osmotic imbalance caused by induced pore and ion channel formation (Fira et al., 2018). Most studies involving bacteriocins have focused on elucidating their antifungal effect against genera such as *Fusarium*; these peptides show great promise in biological control over fungal phytopathogens. Fungitoxic activity usually involves permeabilization of spores or conidia causing damage to the membrane resulting in the inhibition of germination or hyphal cell perturbation (Fira et al., 2018). A particularly interesting interaction is that between *Bacillus* species and plant growth-promoting (PGP) rhizobacteria, which can induce systemic immunity or synthesis of bacteriocins as well as more positive growth-promoting effects; these include synthesis of phytohormones and facilitation of absorption and utilization of certain mineral nutrients (Fira et al., 2018). However, more research is needed on control of bacterial pathogens in this manner, and antimicrobial lipopeptides have no effect on viral strains (Fira et al., 2018). Regardless, the use of biotechnology to isolate or even construct strains which possess biocontrol mechanisms such as antimicrobial lipopeptides as well as plant growth promotion factors is a defense strategy which focuses on marketability of products to growers. Streamlining of the isolation or manufacturing processes to make these products readily available is a worthy focus

for the scientific community when considering strategies for combating the malevolent use of plant pathogens.

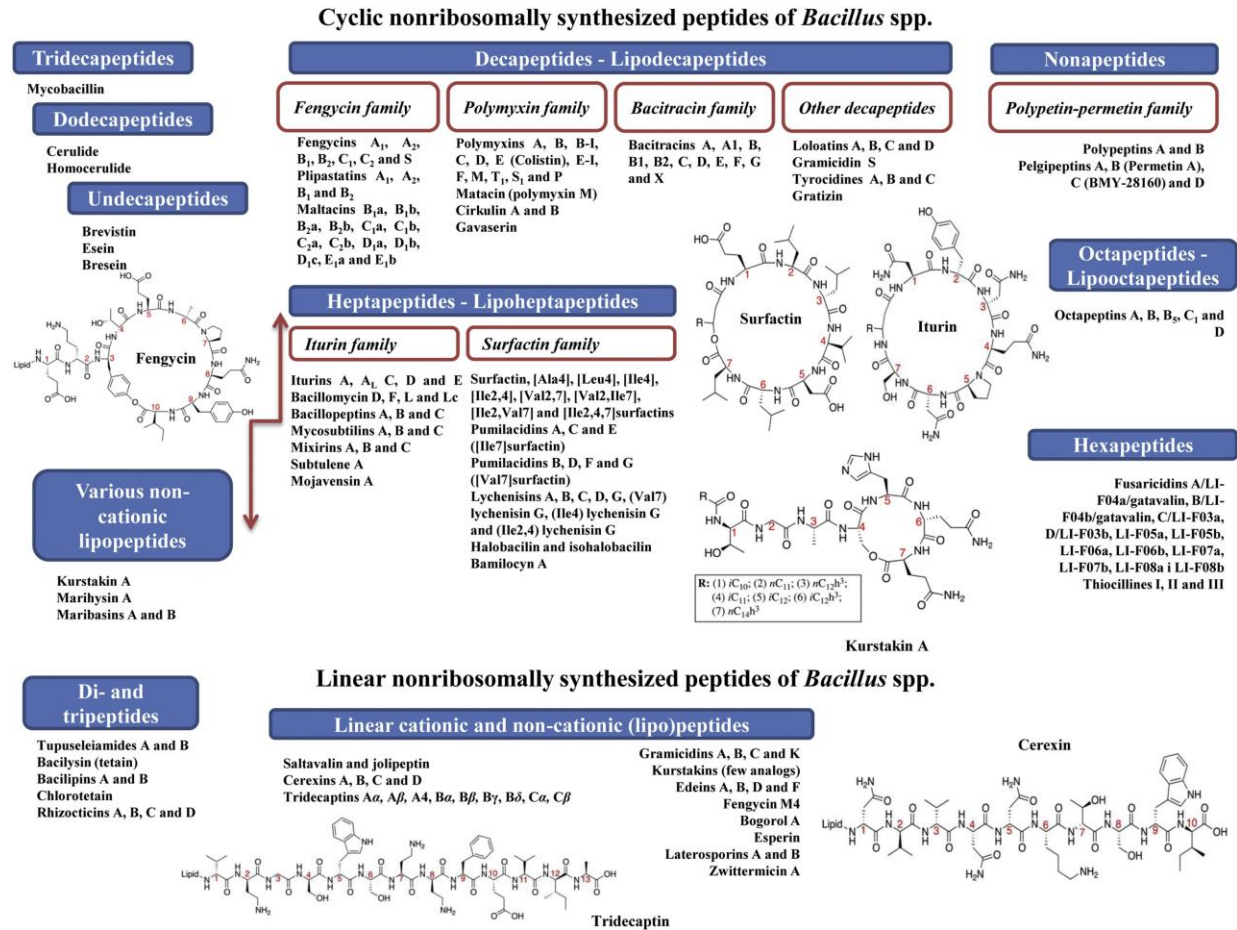


Figure 3. A compilation of cyclic and linear non-ribosomally synthesized peptides containing antimicrobial characteristics; these molecules may be utilized in biosecurity plans intended to prevent fungal invasion of staple crops (Fira et al., 2018).

Autophagy

Plant resistance may also be impacted by the activation of autophagy, as several factors correspond with viral clearance upon induction of the autophagic response. For example, viral particles are frequently found in vacuoles; this supports the idea that there must be an active process that is removing these viruses from the cell (Clavel et al., 2017). An ATG8 protein required for autophagosome formation within the cell was found as an interactor with a virulence

factor of the Cotton Leaf Curl Multan virus and upregulation of genes known to be associated with autophagy, such as *NBR1* (Clavel et al., 2017). Activation of autophagy has been shown to enhance resistance to DNA-containing viruses not only by the formation of autosomes, but also through enhanced autophagic flux and degradation of the capsid protein and viral particles (Clavel et al., 2017).

Conclusion

Attack on agricultural development is a terroristic activity which has existed throughout history as a cause of socioeconomic upheaval and drastic reduction in resources for affected nations. The threat of plant-based agroterrorism continues to grow as the ability of terror organizations to mount expensive, technologically advanced attacks declines due to elimination of leadership and successful counter-terrorism policies. Increase in scientific capability and broader access to information concerning molecular biological techniques and microbial culturing directly relates to the unique threat posed by agroterrorism and needs to be addressed within multiple segments of society. As with any counter-terrorism initiative, involvement of the community is vital to the success of prevention measures. The agricultural and scientific industries are in a unique position to aid intelligence and law enforcement agencies through proper recognition of threats and developments within biotechnology to engineer plant resistance to common and novel plant pathogens. Although the multitudinous steps required to get from seed to table provide a myriad of entry points for infectious agents, proper awareness and implementation of plant resistance strategies will lead to enhanced economic security for humanity's agricultural resources.

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