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Pest Management: The historical management and developed resistance of Western Corn Rootworm, Insect Resistance Management, and the future of control

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Abstract

Western corn rootworm, *Diabrotica virgifera virgifera* Le Conte*,* is a corn pest in the United States that has demonstrated overwhelming success an overcoming pest management strategies. Whether it is cultural, chemical, or transgenic control, western corn rootworm has managed to adapt, develop resistance, and create chaos among farmers and agriculture industries. Insect Resistance Management is a component of the Environmental Protection Agency and the Excellence Through Stewardship global organization. The Environmental Protection Agency regulates resistance through laws and regulations and with the help of other government organizations. The goal is to delay resistance, extend trait durability, and protect the environment and human health. The industry coordinated organization, Excellence Through Stewardship, helps with these goals by providing insect resistance management guidelines to agricultural companies and growers. With the development of resistance to control methods, there are several advancements in technology that have allowed for new and improved methods for insect control in agriculture. These new technologies will optimistically provide relief for resistance against other pest control methods, protect the environment, protect human health, and continue to provide food for our rapidly developing world.

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I. Prologue

The choice for my degree project was very broad due to the major applications to my previous and current role within the agricultural industry. I have worked for an agricultural company for six years and have a diverse background in the laboratory, greenhouse, field, quality, and compliance. My work specific work experience includes working in an entomology bioassay and discovery lab as a research associate and currently as a senior quality and compliance analyst. My first role included working with agricultural pests and performing high-throughput artificial diet bioassays, insect rearing, insect resistant assays, greenhouse testing, and field testing where a majority of my work was focused on western corn rootworm. The discovery research work that I have done with my current company has been focused on western corn rootworm, specifically RNA interference and Bt. My current role as a senior quality and compliance analyst requires an understanding of the research process from discovery to production of material. In this role, I perform quality and compliance audits as well as internal Excellence Through Stewardship and Insect Resistance Management Audits. Through my education at University of Nebraska-Lincoln, I have been able to focus on entomology, expand my knowledge in this area, and apply this knowledge to my role in agriculture. There are several emerging technologies, specifically CRISPR and RNA interference, that are being developed for use in pest control and I feel that my diverse experience, education, and my master's project provides a background of pest management, IRM programs, and an insight into the future.

II. Introduction

Insects are one of the most abundant and fascinating group of animals on the Earth. While many insects are beneficial to humans and the environment, some are harmful and devastate our crops that we depend on for food and money. Insects compete with humans in several different ways from crop devastation to horticulture and forestry and the term pest has developed due to this competition for resources. Pests can be threats at all levels and include both low and high densities. Pest management is necessary to control pest populations and avoid significant losses for farmers. Insect pests of agronomic crops cause billions of dollars in damage each year. Historically, pest management involved the use of cultural, chemical control, and the use of transgenic crops such as Bt. The increase and overwhelming ability for pests, particularly western corn rootworm, to adapt these control methods has led to a global disarray and the increased need for new methods.

Western corn rootworm, *Diabrotica virgifera virgifera* Le Conte, is primarily a corn pest that is estimated to cause one billion dollars in damage each year (Gray et al., 2009). Western corn rootworm has mainly been controlled using crop rotation management and this proved to be an effective control method for decades (Gray et al., 2009). Then in 1995, there was an increase in root damage noted in regions that had been utilizing crop rotation (Gray et al., 2009). This was an incredible behavioral adaptation in a short time period. Of course this would not be the last example of resistance in western corn rootworm. Resistance to pesticides was also developed with the use of DDT. With the rapid deployment of transgenic plants, it was only a matter of time before resistance was developed to transgenic crops.

The continued exposure to crop rotation, increased and spontaneous use of chemical control, and the increased deployment of transgenic crops, has led to an overwhelming increase in resistance from several insect pests. This has led to farmers and agricultural companies scrambling for new technologies and forced governmental agencies to increase regulations on the biotechnology industry. The alarming rate that insects have achieved resistance led to the need for Insect Resistance Management strategies. Insect Resistance Management strategies have been developed to help with resistance control which includes the incorporation of Integrated Pest Management techniques. Integrated pest management is an integral approach to management. The ultimate goal is to decrease pressure caused by resistant pest populations while considering the biology of the pest, the environment, adverse health effects, and safety concerns.

This literature review will provide a journey throughout history from the first control methods used for pest control, and introduce the concept of resistance and provide insight on how we got to where we are today. This paper will also provide the framework of Insect Resistance Management (IRM), and requirements for IRM programs. I will also explain the need for Integrated Pest Management (IPM) and the movement of the EPA towards an integrated approach. Finally, I will provide a look into the technology of the future and emerging technologies that are providing breakthroughs for the industry. This paper will concentrate on Western Corn Rootworm, *Diabrotica virgifera virgifera* Le Conte.

III. Pest Management

Western Corn Rootworm

The western corn rootworm has been an economic pest in the United States for decades and are commonly grouped together with the norther corn rootworm species. The western corn rootworm is from the order Coleoptera and in the Chrysomelidae family. Western corn rootworm is native to the United States and the distribution is mainly through the Corn Belt region of the Midwest and expands eastward. The range has also been expanded to Europe due to unintended introduction. The western corn rootworm undergoes complete metamorphosis with an egg, larval, pupal and adult stage. There is one generation per year. Adult beetles emerge in June, mate, and the females can lay up to 500 eggs in August.

Western corn rootworm feed on the roots, leaves, silks, and kernels or corn plants. The damage occurs in both the larval and adult stages. The majority of damage is caused by the larval stage that feed on the below grown parts of the plant immediately after hatching. Figure 1 shows a comparison of healthy corn root system versus a destroyed corn root system from western corn rootworm larval feeding (Head, Covett, n.d.). The larvae tunnel through the root system which decreases yield and causes lodging of the corn stalks. The adults are the less devastating stage but do feed on silks, kernels, and leaf tissue. Once the silks emerge, the adults feed on the silks which may decrease fertilization. The adults also feed on the kernels of the exposed ear and the exposure increases the chance for pathogens.

Figure 1 (Head, Namuth-Covert, n.d.)

Control Throughout History

Pest management strategies have been used since crop cultivation began. Original methods were focused on available resources such as sulfur, physical barriers, mechanical control, or field burning to remove pests. New methods were developed throughout history as technology progressed. Control of insects has historically paid little attention to the overall effects on the environment, pest populations, and non-target organisms. Rather, the focus was on how cheap the control was, the effectiveness, and how fast it was going to work. One of the main goals of early pest management was the need for immediate control of pest populations. There was limited knowledge of the biology of the pest, lack of concern for environmental, human health, and other downstream effects.

There are several types of control methods for pest populations that have been used since the beginning of agriculture. More modern day control methods include cultural, biological, physical, host resistance, transgenic, and chemical control. Methods have evolved over time and some methods work for one pest but not another due to the diversity of insects. This stresses the importance of understanding the biology of the pest in order to determine the best control method. The western corn rootworm has historically been controlled through cultural control and crop rotation with soybeans, chemical control, and the use of transgenic crops.

Cultural Control

Cultural control is one of the oldest control methods that involves environmental manipulation that decreases favorable conditions for the pest. Cultural control essentially disrupts the relationship between the pest and the host crop. Examples of cultural control include tillage, crop spacing, crop fallowing, crop location, and crop rotation. Crop rotation was initially imposed for soil improvements but resulted in a useful pest management strategy. Crop rotation is most successful against pests with fairly immobile life stages, narrow host feeding range, and low survival over long periods of time when in the absence of food (Onstad, 2014).

Crop rotation was the most common and successful historical form of control against western corn rootworm for several decades.

The USDA reported in 2003 that 25% of corn grown in the United States was involved in crop rotation of some kind (Onstad, 2014). The lack of a rotation led to western eggs that were laid the previous season emerging to the crop they were laid in and hypothetically the crop of choice for the pest. Crop rotation led to larval emergence in an alternate crop that the eggs were initially laid in. Crop rotation disrupts the lifecycle and causes starvation of the pest because the larvae are weak and unable to travel long distances to reach suitable food. This control method is environmentally safe, economical, and has several benefits beyond pest management. Crop rotation decreases the need for other control methods such as chemical control.

Chemical Control

Chemical control includes insecticides, pesticides, herbicides, and fungicides. Chemical control of pests began almost simultaneously with crop cultivation itself. The use of chemical control can be traced back to 2500 B.C. and the use of sulfur to control pest populations (Pedigo, Rice, 2015). Pesticide use ranged throughout history from the use of soap, to arsenic, and nicotine. There was an expanded use of insecticide application in the early 1900s as technology allowed the development of new and improved types of chemicals. Chemical control was often used for the immediate effects that it would have on the pests.

Chemical control was used heavily for control beginning with the use of DDT in 1944 and thus this time period earned the name, the insecticide era (Speight, 2009). The insecticide era ranged from 1939 to 1962 and involved the use of heavy of chemicals that provided extraordinary control of pest populations (Pedigo, Rice, 2015). The insecticide era involved repeated insecticide applications with little awareness to the ecological effects and long-term resistance problems that would arise. This concept is termed the pesticide treadmill because of the continuous cycle of repeated applications that meant more problems which resulted in more pesticide applications. This resulted in a treadmill effect that was a downward spiral spinning out of control. DDT is a synthetic insecticide that was used to control malaria and other diseases during WWII. DDT seemed to be a success and saved lives, but it was then found in milk which started the safety concerns of human health and the environment. The book, *Silent Spring*, was also published around the same time in 1962. The book brought awareness to the negative effects of chemical use and brought the issues of safety and health to the attention of the public.

Despite the recent development of public concern, pesticides have been used throughout history as a reliable control method. Pesticides, more specifically insecticides, are used to destroy, repel or mitigate pests. There are several types of application methods and active ingredients. Pesticide control for western corn rootworm can occur at several different times during the lifecycle of both the pest and crop stage. The application of chemicals depends on several factors related to pest population density, insect damaging stage, and timing of planting. Granular insecticides may be used simultaneously with planting to target early instar larvae. Aerial spray insecticides can be a method for large fields and high density populations. Aerial applications can also be used to target gravid females. Foliar sprays are used to prevent adult western corn rootworm feeding on the exposed silks. There is a wide variety of pesticide available for use that are often concoctions that contain multiple treatments within one application.

Transgenic Control or Plant-incorporated Protectants

Transgenic control involves the use of transformed plants. These transformed plants have been genetically engineered and are often referred to as genetically modified organisms or GMOs. Plants are transformed through biotechnology and the insertion of a gene into the plant. The United States Environmental Protection Agency (EPA) uses the term plant-incorporated protectants, or PIPs, to describe transgenic plants. PIPs are defined by the EPA as, "…pesticidal substances produced by plants and the genetic material necessary for the plant to produce the substance." (EPA, n.d.).

The most common gene is the cry gene that produces an insect pathogen and is often referenced as Bt or *Bacillus thuringensis*. Bt is a rod-shaped microorganism that are naturally occurring in the soil. BTs have been used for over 70 years in agriculture in the form of control (Onstad, 2014). Bt microorganisms are composed of living spores and an endotoxin. Toxins are produced by Bt and expressed in the plant. The larvae then feed on the corn and ingest the toxin. The activated toxin then penetrates the gut lining and attacks the cells. This ultimately leads to larval mortality due to spores and bacteria flowing through the holes in the gut and into the larval body (Glaser, Matten, 2003). Mortality usually takes a few days to weeks, but the effects ultimately deter the insect from continuing to feed on the plant material due to the weakened state of the insect.

The use of transgenic corn in the United States is 67% of all corn planted as of 2012 and the planting of transgenic crops continues to rise (Cullen et al., 2013). A Key component of Bt crops is the planting of non-Bt refuge seed in a range of five percent to 20 percent and is dependent on several factors (Onstad, 2014). Bt crops provide many environmental benefits, they are known for the ability to target a narrow host range, and commonly have low exposure rates. The biggest environmental benefit that Bt crops offer is the reduction or elimination of chemical control. A three-year survey of US corn growers using Bt corn had a 200% reduction in the use of chemical control (Glaser, Matten, 2003). There is also a reduction in the non-target effects when using Bt crops due to the ability to provide a narrow target of the pest. With the growing concerns for non-target organisms such as bees, Bt crops offer a safer approach and also please the grower with increased yields and other benefits. GMO crops are getting a lot of attention due to the perception that GMOs may be harmful. The release of GMO crops was immediately received as negative and agricultural companies were being portrayed as evil. GMOs are very controversial and the related, or possibly unrelated depending on who you ask, negative effects often outweigh the significant benefits GMOs provide. This topic is a paper within itself, but it is important to point out the growing concerns.

Development of Resistance

Causes and Mechanisms

In order to outline the causes and mechanisms of resistance, it is necessary to define resistance. Onstad defines resistance as, "…evolutionary changes in an insect population that occur in response to repetitive exposures to insecticides or other xenobiotics used to manage insect pests in crops, homes, gardens, or on livestock or humans." (Onstad, 2014). The definition can be simplified to a sensitivity reduction of an inset population (IRAC, n.d.). Onstad also adds that resistance can be broadened to include naturally occurring abiotic and biotic factors that insects encounter in the environment (Onstad, 2014). Natural selection also plays a role in resistance. Natural selection allows for resistance traits to be passed on, and eventually the resistant insects greatly outnumber the susceptible. The rate at which an insect develops resistance depends on genetic, biological and operational processes (Storer, n.d.). Pests of agricultural crops, western corn rootworm in particular, have demonstrated their ability to overcome management practices and develop resistance to almost every management strategy deployed against them.

Historically employed control methods are no longer sufficient for control of insect pests due to the ability of insects to persist and consistently thrive against them. The repeated crop rotations, increased use and rapid deployment of transgenic crops, and over exposure of insecticide applications, has led to the ultimate development of resistance and resurgence of pest populations. Western corn rootworm has historically been distinguished as a corn pest which is why crop rotation to soy had been a successful management strategy for both larval and adult feeding stages.

The first western corn rootworm adaptation to crop rotation was recognized in 1987 in Illinois (Onstad, 2014). There were several hypotheses developed around the adaptation that included the repellency hypothesis, and isolated instances. These were disputed when growers were suffering incredible losses in Illinois and Indiana in 1995 (Onstad, 2014). It was concluded that there was in fact a behavioral adaptation and western corn rootworm were laying eggs in adjacent fields of soybean, alfalfa, wheat, and oats (Onstad, 2014). The females that lay their eggs in soybean fields increase the chance for larval survival due to the subsequent crop most likely being corn (Onstad, 2014). This means that the adaptation to crop rotation occurred at a very fast rate and occurred in approximately 17 years.

In recent years, there has been development of a rotational resistant variety that allows for not only egg laying in soybean crops but also feeding on soybean crops. Cultural control was a successful control method for western corn rootworm for decades however, there has been discovery of female westerns laying eggs in soybean fields and even feeding on soybean plants. It was found that western corn rootworm females were laying eggs in soybean fields and that an adaptive strain had been selected. O'Neal et al. did a sampling test and determined that the presence of female western corn rootworm in soybean females was higher when compared to corn (Gray et al., 2009).

Feeding on soybean foliage has been documented in western corn rootworm females and can be seen in figure 2. Figure 2 shows green bodied females on the left that have consumed soy material and yellow-orange bodied females on the right that have consumed corn. This shows that although the soy material is not the preferential choice, the females have adapted to this feeding strategy for egg laying purposes. It is estimated that 60 percent of the resistant western corn rootworms that are found in other fields besides corn are female (Onstad, 2014). In late July in Illinois, sweeps of 200 collected beetles averaged 86 percent female and they had consumed soy tissue (Onstad, 2014). Lab tests using field collected western corn rootworm who are only fed soybean tissue die within one week (Onstad, 2014). Field collected westerns who were given 50 percent corn and 50 percent soy plant tissues were shown to survive as long as those fed corn tissue only (Onstad, 2014). This demonstrates that strictly feeding on soy tissue may not be the only food source but that they are able to feed and survive on a diet of both corn and soy.

Figure 2 (Gray et al., 2009)

The soybean feeding is thought to cause stress and cause behavioral changes that may in fact increase egg production in some cases. In most cases, this requires the female to return to corn tissue for feeding to ensure proper reproduction and egg development. Although crop rotation has led to resistant populations of western corn rootworm, the eggs that are laid and hatch in soybean fields still leads to larval mortality due to the inability of larval migration to an adjacent field of preference. Gray et al. concluded that rotational resistance is a genetic trait but there is not a developed molecular marker associated with this trait at this time (Gray et al., 2009). The development of crop rotation resistance by western corn rootworm was unexpected and it took years for growers to have confidence in in the development. Crop rotation is still recommended due to the inability for larvae to survive on soy roots and especially for areas where rotation resistant western corn rootworm is not present.

Along with resistance development to crop rotation, there was also developed resistance to chemicals. Resistance, in regards to insecticides, is defined by Onstad as “a general term representing heritable traits selected by management.” (Onstad, 214). The heritable traits lead to the ability of an insect to overcome pest management techniques, leaving farmers and scientists to scramble for a method of control. The pesticide era involved the repeated overexposure to insecticides and ultimately led to pest adaptation and resistance. Resistance to pesticides for most insects can be attributed to genetics and the amount of application (IRAC, n.d.). The time it takes to develop resistance depends on the amount of pesticide applications, timing of applications, the reproduction rate, host range, migration ability, and specificity (IRAC, n.d.)

There are several mechanisms of resistance to insecticides in pest populations. The mechanisms of resistance include reduced penetration, increased sequestration or excretion, behavioral resistance, metabolic resistance, target site sensitivity, and cross-resistance (Onstad, 2014). Reduced penetration resistance involves a heritable mechanism that reduces or even prevents the entry of toxins upon feeding (Onstad, 2014). Increased sequestration mechanisms involve the binding of an insect's proteins or enzymes bind with the insecticide molecules and push the bonded material away from the target site (Onstad, 2014). Behavioral resistance is when an insect deploys an avoidance behavior in order to survive or increase chances of survival (Onstad, 2014). Metabolic resistance is the ability of an insect to speed up their metabolism to break down insecticides at a higher rate (Onstad, 2014). Target site insensitivity mechanism decreases the toxicity of the insecticide and includes cross-resistance (Onstad, 2014). These mechanisms are utilized by insects and the specific mechanism varies between species.

Bt corn was available to growers in the United States commercially in 2003 and the amount of Bt corn planted over the next few years was enormous. It is estimated that of corn planted in the United States in 2012, 67 percent had at least one Bt trait (Cullen et al., 2013). The increased planting of Bt corn in a short period of time placed huge selection pressure on pests and ultimately led to resistance by western corn rootworm. Explanation of resistance to Bt can be attributed to the increased pressure, the lack of compliance to refuge, the use of single traits in a continuous pattern, and detoxification mechanisms (Onstad, 2014).

Fields that planted the same Bt trait continuously over multiple years, have seen developed resistance due to the selection pressure placed on the pests. Fields have also seen resistance to Bt traits where refuges are not being fully utilized or implemented properly. A refuge ensures that insects feed, mate, and reproduce on non-Bt material and ensures that the susceptible gene is passed on to subsequent generations. If refuge is not planted, the insects will develop resistance at a higher rate. Additional explanations for resistance include the ability of insects to alter the protein process and reduce the toxicity through detoxification (Onstad, 2014). There is also evidence of modifications of midgut enzymes and binding sites (Onstad, 2014). The ability of an insect to detoxify an ingested protein reduces the toxicity and thus increases survival.

There are several mechanisms and reasons as to why insects develop resistance. The increase in resistance and increase in time it takes for a pest to become resistance has led to a need for resistance management. The ability for western corn rootworm to continuously develop resistance to crop rotation, pesticides, and *Bt* has led to the amplified involvement of government agencies and ultimately the formation of Insect Resistance Management (IRM) programs. Resistance is a global issue that is not only attributed to western corn rootworm in the United States. Resistance is found all over the world with multiple pests. This makes IRM a global issue that requires several steps that include proper management and consistent monitoring.

IV. Insect Resistance Management

Insect Resistance Management (IRM) is defined as strategies or practices that target the reduction of resistance in insect pests (Glaser, Matten, 2003). The management of resistance is complex and requires understanding of the evolution of pests and the evolved pests value (Onstad, 2014). The goal of IRM is to delay the evolution of resistance and prolong the lifespan of products. IRM plans are put in place to decrease the chance for developed resistance through regulations that include registrations, testing, refuge, and other pest management practices. IRM is mandated by the EPA in order to preserve beneficial management strategies and delay resistance. IRM is also a component of the Excellence Through Stewardship organization and provides guidance for resistance management and programs.

EPA

The Environmental Protection Agency (EPA), U.S. Department of Agriculture (USDA), and the Food and Drug Administration (FDA), all play integral roles in resistance management. These organizations are responsible for addressing the challenges by implementing rules and regulations for the use, sale and distribution of pesticides and PIPs for use in pest management. Pesticides include synthetic chemicals, naturally occurring biochemical, microbial agents and PIPs (Wozniak, Martinez, 2011). The EPA is ultimately the responsible agency. The EPA follows specific laws for registration, compliance, and regulations for agriculture in regards to pest management practices.

The EPA was established in 1970 with the overall goal of protecting the people from pollution. The EPA has developed a mission statement to include the protection of not only human health as initially stated, but also the environment. The EPA involvement in pesticides came about with DDT and the acquisition of pesticide registrations from the USDA under the 1964 Federal Insecticide, Fungicide, and Rodenticide Act (Williams, 1993). The EPA was ultimately responsible for the ban on DDT. The EPA has broad focus on the environment but this paper will focus on the agriculture and biotechnology portion.

Pesticide Regulation

The EPA defines a pesticide as a substance or mixture that is used for the following: preventing, destroying any pest, plant regulator, defoliant, desiccant or as a nitrogen stabilizer (United States Environmental, n.d.). In regards to regulation of pesticides, the EPA regulates under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetics Act (FFDCA). The FIFRA involves all legal requirements for the registration of all pesticides and also genetically modified plant parts that are resistant to disease (United States Environmental, n.d.). FIFRA states that all pesticides in the United States intended for sale or distribution are required to be registered through the EPA (United States Environmental, n.d.). FIFRA also requires several steps before approval including proof that the pesticide will not cause adverse environmental effects (United States Environmental, n.d.). The FFDCA focuses on pesticide residues on food and animal feed and requires set tolerances to be in place through the EPA (United States Environmental, nd). The EPA must then evaluate the pesticide for risks to the environment and health and this is achieved by setting tolerance levels for pesticides. The EPA specifically evaluates risks to human health, non-target insects, probability of gene flow, and IRM plan needs.

The pesticide registration process involves evaluation of ingredients, area of location the pesticide is planned to be used, the amount to be used, frequency of use, the method of storage, and details of disposition of the pesticide (United States Environmental, n.d.). The evaluation portion of the registration process involves the assessment of human health risks, environmental risks and includes a risk assessment of scientific data and risk management and regulatory conclusions (United States Environmental, n.d.). The label must also be reviewed by the EPA before distribution to ensure that the directions for use are clear and concise (United States Environmental, n.d.). There is also a compliance portion where users must comply with state and federal laws for pesticide application (United States Environmental, n.d.). Compliance to the laws in monitored through inspections, investigations, and data collection (United States Environmental, n.d.).

The EPA also has a risk assessment portion that evaluates the ecological, human, and cumulative risk of pesticides (United States Environmental, n.d.). This is used for both new and existing pesticides. Existing pesticides are required to be evaluated to safety standards to ensure that they are still meeting the specific safety requirements as initially stated. The ecological risk assessment is responsible for making sure that non-targets such as plants, fish, and other wildlife are not exposed to unreasonable risk (United States Environmental, n.d.). There are four phases to the process that involves a planning phase, problem formulation phase, analysis phase, and risk characterization phase (United States Environmental, n.d.). The risk characterization phase has two components. The first component is the risk estimation phase and the second component is the risk description phase (United States Environmental, n.d.). The risk estimation component defines the exposure profiles and provides information as to what the exposure effects are (United States Environmental, n.d.). The risk description component details information and levels of harmful effects (United States Environmental, n.d.).

The human health risk assessment is a four step process that evaluates the possibility of adverse health effects. The first step is hazard identification to evaluate the level of harm to humans (United States Environmental, n.d.). The second step is a dose response to assess the rate of exposure to the effects (United States Environmental, n.d.). The third step is an exposure assessment followed by the fourth step of risk characterization (United States Environmental, n.d.). There are several details to this process to ensure the safety of humans if exposed to a pesticide. The cumulative risk was implemented due to the increase in the toxicity mechanisms of pesticides. This risk assessment is used to gauge the associated effects with exposures from a group of chemicals (United States Environmental, n.d.). There is specific data information that is required for registration that is detailed in 40 CFR Part 158 (United States

Environmental, n.d.). The 40 CFR Part 158 are regulations that provide registration decision information to the EPA and allows the EPA some flexibility in decision making in regards to what is important for the specific registration (United States Environments, n.d.). There are also specific testing procedures that are required by the Toxic Substances Control Act and FIFRA (United States Environmental, n.d.). All the requirements have been constructed into guidelines that meet requirements by all involved parties.

PIP Regulation

The EPA registered the first Bt PIP in 1995 (United States Environmental, n.d.). As of 2016, 100 PIPs have been registered between corn, cotton and soy crops (United States Environmental, n.d.). The EPA regulates the gene rather than the plant itself. The EPA has five principles for decision making in regards to biotechnology These five principles include using sound science, transparency, consistency and fairness, collaboration with regulatory partners, and building trust with the public (United States Environmental, 2017). In 1986, the Coordinated Framework for the Regulation of Biotechnology was passed by the Federal Government in regards to the regulation of biotechnology products (The United States Environmental, n.d.). Coordinated Framework ensure that there is still some flexibility in research and that there are no new laws required for regulation of biotechnology products (United States Environmental, n.d.). Regulations would still be required through the EPA and the framework requires environmental approval and food safety processes by the USDA, FDA, and EPA (United States Environmental, n.d.).

The EPA requires IRM plans for Bt crops because they are pesticide substances produced by plants. The EPA uses the term plant-incorporated protectants (PIPs) as identification for the unique biotechnology class that includes substances produced by plants for protection, Bt cry proteins, and the genetic material, such as *cry* genes, required for production in the plant (Mendelsohn, 2016). Resistance management of PIPs is necessary due to the risk of resistance, the need to preserve the benefits, and PIP resistance is considered by FIFRA to be an unreasonable adverse effect (Reynolds, 2016). The EPA began registering PIPs under FIFRA and FFDCA in 1994 under proposed rules of plant-pesticides (United States Environmental, n.d.). The EPA then requested reevaluation by the scientific advisory board in 1999 due to the overall decrease in pesticide use with the use of PIPs. The EPA published rules that exempt PIPs from FIFRA requirements and FFDCA residue requirements in 2001 (United States Environmental, n.d.).

There is a considerable amount of information necessary to develop a resistance management strategy for PIPs. The information necessary includes the resistance management risk assessment, full understanding of the pest biology, the dose of the PIP, the resistance mechanisms, possibility of cross resistance, simulation modeling, and mitigation strategies (Reynolds, 2016). There is also a general stewardship model for post registration and the activities necessary. These activities include resistance monitoring, a strategy for remedial action, refuge program, an education program, and reporting to the EPA (Reynolds, 2016). The resistance monitoring portion is necessary for the target pest in order to determine the field damage and changes in susceptibility (Reynolds, 2016). This can be done through sampling techniques and must focus on the geographic regions of the pest.

The remedial action plan provides steps to take if resistance develops in the pest population. Refuge is part of the compliance aspect of IRM and may include a structured or blended refuge (Reynolds, 2016). It is important to have grower agreements in place to ensure that the proper refuge strategy is being implemented. The education component is vital to the resistance management plan because the growers are in contact with the fields on a day to day basis. It is important to educate growers in the areas of compliance and resistance to ensure that the plan is being followed. The growers need to know why they have to do additional steps and why it is important and this is a key component to success. The final component of the stewardship plan is the reports to the EPA. The annual report to the EPA should include all of the components under the stewardship model.

The main cause of field-evolved resistance to Bt corn by WCR was due to the inadequate refuge planting (Cullen et al., 2013). This ties into the educational component for the growers. The EPA recommendations vary according to the PIP registered and the planting region. The refuge model is based on the biology of the European corn borer and the high dose structured refuge strategy. The goal of the high dose structured refuge strategy is to use a high dose Bt and refuge with non-Bt to allow for mating with resistant insects and susceptible insects. The goal is to decrease the chances for development of resistant alleles in the population (Bourguet et al., 2005).

The Corn Belt region of the United States has different refuge percentages compared to the southern part of the United States due to the population pressure differences and growing differences. The two types of refuge strategies, structured and seed blend, vary in amount. The structured refuge for a single trait should be twenty percent and the pyramid traits should be at five percent (Reynolds, 2016). The seed blend for a single trait should be at ten percent and the pyramid trait should be at 5% (Reynolds, 2016). There have been several recommendations made to help resolve or decrease WCR resistance to *Bt* corn. Some of these recommendations include crop rotation, long-term approach to management, elimination of point sources, and the use of Bt stacks or pyramided Bt hybrids (Cullen et al., 2013). The EPA now requires contracts with growers to ensure compliance with the refuge amounts. As discussed previously, the contracts ensure that refuge requirements are followed and noncompliance may lead to fines or the inability to utilize the traits for future plantings. The fines enforce the importance of the refuge requirements and that there will be consequences if the rules are not followed.

The registration process for PIPs requires several steps and fees before a product can be registered. There are several types of PIPs that have been registered and include single trait, stacked and pyramid traits, seed blends, commercial use, old active and new active ingredients (Mendelsohn, 2016). The first step is to consult with the EPA during research and development of a product. The next step is to obtain an Experimental Use Permit (EUP) which allows for data generation and the EPA also calculates the inert and active ingredients for the PIP (Mendelsohn, 2016). A EUP is required when ten acres or more are being used for testing and several steps must be taken to prevent out-crossing of pollen from the PIP (Mendelsohn, 2016). The requirements for corn include spatial, reproductive, and temporal isolation (Mendelsohn, 2016). The EUP includes a fee, application process, description of areas the product will be used, amount used and several other details relating to the program (Mendelsohn, 2016).

The EPA evaluates the active ingredients and products for identification of a PIP. There are five components under evaluation that include the active ingredient, inert ingredients, transformation event, the vector, and the plant itself (Milewski, 2016). These components play a role in the registration process and whether there is a new active ingredient being registered with a new product or a new product with an already registered active ingredient. There are several aspects that the EPA considers in the registration process for PIPs just as with pesticide registrations.

Registration Review and Fees

The registration process for pesticides and PIPs can be extensive and can take years to complete. With this lengthy process comes numerous associated fees. The Pesticide Registration Improvement Act (PRIA) regulates the fees for each step of the process. PRIA was proposed by stakeholders, industry representatives, and non-governmental organizations and was passed by congress in 2004 (Wozniak, 2012). There are several review phases for registration that begin with a 21-day consent screen to determine if all required information has been submitted with the application (Mendelsohn, 2016). The preliminary technical screen follows and checks for accuracy of data, consistency of labeling and tolerance levels (Mendelsohn, 2016).

The PRIA fees ensure that decisions around the registration process and decisions are completed in a particular timeframe. There are also associate maintenance fees. In 2016, the annual fee for each registration was $3,472 (Mendelsohn, 2016). The maintenance fees may be reduced for smaller businesses and minor registrations. The timelines and fees depend on the complexity of the registration. There are cost differences between registering for a new product or active ingredient, obtaining a EUP, or amending a previous registration. For example, obtaining an Experimental Use Permit can take up to six months of review and cost $95,724 for the service fee (United States Environmental, n.d.). Registration for a new PIP can cost up to 400,000 and take anywhere between 18 and 24 months for review depending on the type of PIP registration needs (United States Environmental, n.d.). An example of PRIA timelines and fees for PIPs can be seen in figure 3 (Mendelsoh, 2016).

Examples of PRIA Timeframes and Fees for PIPs

PRIA Code	Regulatory Action	Decision Review Time in Months	FY'16/17 Registration Service Fee in $
B771	Experimental Use Permit application; new PIP; with petition to establish a temporary tolerance/tolerance exemption for the active ingredient; credit 75% of B771 fee toward registration application for a new active ingredient that follows.	10	127,630
B780	Registration application; new PIP; non-food/feed.	12	159,537
B820	Registration application; new PIP; with petition to establish or amend a permanent tolerance/tolerance exemption of an active ingredient.	15	319,072
B880	Registration application; registered PIP; new product or new terms of registration; additional data submitted; no petition since a permanent tolerance/tolerance exemption is already established for the active ingredient(s).	9	31,910
B900	Application to amend a registration, including actions such as extending an expiration date, modifying an IRM plan, or adding an insect to be controlled.	6	12,764
B904	Import tolerance or tolerance exemption; processed commodities/food only (inert or active ingredient).	9	127,630

Figure 3 (Mendelsohn, 2016)

<u>Framework to Delay Resistance</u>

The EPA recently published an article that addresses western corn rootworm resistance. The article provides new and improved requirements to delay resistance to Bt corn. This framework was approved for the 2016 growing season with the goal of prolonged durability, maintained effectiveness of traits, and management of resistance (Reynolds, 2016). The framework to delay focuses on several components and requirements. The focus remains on stewardship and IRM plans that ranges from IPM tools, and grower education. There is also a standardization component for unexpected damage and sampling of insects and testing in bioassays (Reynolds, 2016). The framework also eliminated a requirement of random sampling in the corn belt region due to the lack of meaningful data. (Reynolds, 2016). Details around remedial action plans and how to contain resistance and maintain trait durability have been further developed (Reynolds, 2016). Emphasis is placed on scouting in areas of unexpected damage and communication with the Bt corn supplier (Reynolds, 2016). This article is meant to help alleviate the stress of resistance and provide beneficial information.

<u>Excellence Through Stewardship</u>

Agricultural companies have joined together to discuss similarities of products and the idea of licensing traits from companies. The members of CropLife International, BASF, Bayer Crop-Science, Dow AgroSciences, DuPont Pioneer, Monsanto, and Syngenta, came together due to their similarity of products and the global pest resistance problems that the companies are all facing (Storer, n.d.). The companies made a commitment to stewardship of technology that includes resistance management practices across all parts of organizations. The organizational parts range from research and development, regulatory and commercial business, to technology sustainability (Storer, n.d.).

The Excellence Through Stewardship (ETS) organization was created in 2008 to support industry efforts and allow input from agricultural companies for practical management. ETS is a global organization that focuses on the responsible management of biotech plant products, the use of quality management systems, and the implementation of stewardship programs for all stages of the product lifecycle (Storer, n.d.). The resistance management components of ETS were added in 2015 with the expectation that members would undergo an ETS audit by the end of 2016 (Storer, n.d.). The ETS guide for resistance management provides direction for the development of IRM programs. The ETS guide for IRM, seen in figure 4, provides guidance on IRM plans for traits, alignment of the industries on IRM strategies, availability and distribution of refuge seed to growers, adoption of IRM programs by growers, and monitoring for effectiveness of the IRM program (Storer, n.d.).

Figure 4
(Excellence Through Stewardship, n.d.)

The guide is divided into four modules that includes risk assessment, IRM plan development, IRM plan implementation, and IRM program maintenance and compliance with requirements (Excellence Through Stewardship, n.d). The risk assessment module involves a risk assessment of resistance to traits in regards to the location where the trait will be produced and commercialized (Excellence Through Stewardship, n.d). The organization should determine who is responsible for the IRM program and serve as the subject matter expert (Excellence Through Stewardship, n.d). Identification and assessment of the planting location is important for determining the appropriate IRM plan. The IRM plans can vary by geography due to the size and distribution of the land, crop rotation, historical deployment of genetically engineered crops, and compliance with refuge requirements (Excellence Through Stewardship, n.d). The proper pest identification and understanding of the pest biology is necessary in order to determine the scope of the IRM requirements (Excellence Through Stewardship, n.d). Aspects of the pest biology and life cycle that should be taken into consideration include the number of insects, assessment of damaging stages, migration, and history of resistance (Excellence Through Stewardship, n.d). Procedures should be established in the risk assessment portion to ensure that the appropriate records and documentation are being followed and retained.

Module two is the IRM plan development that should capture the information from the risk assessment. The risk assessment is comprised of evaluation of the trait performance, establishment of baseline susceptibility for the target pest, development of IRM requirements that are based on historical management practices, refuge evaluation, evaluation strategies for effectiveness, stakeholder communication, and procedures for documentation and records. Module two emphasizes the importance of research and planning. It is important to develop pest monitoring and sampling methods and evaluate refuge requirements. Refuge requirements are based on several factors from industry guidance and regulatory requirements and the deployment options for the refuge seed. The emphasis is also on evaluation of the program itself. This involves assessment of the program effectiveness in regards to resistance monitoring, product performance, compliance of the grower, and education and training of growers and sales representatives (Excellence Through Stewardship, n.d).

Module three is the implementation phase of the IRM plan into the business activities. This step highlights training of involved parties and communication of the IRM plan. The education component is very important to ensure compliance in all aspects of the IRM program. Communication and training should include growers, stakeholders, distributors, licensees, and employees (Excellence Through Stewardship, n.d). The IRM program maintenance and compliance is found in module four. The establishment of monitoring procedures for use patterns, compliance with refuge and other requirements, and resistance monitoring are all necessary components of maintenance plans (Excellence Through Stewardship, n.d). It is also necessary to have plans in place for dealing with growers who do not follow the refuge requirements and to consider what needs to be reported to regulatory for resistance and compliance monitoring (Excellence Through Stewardship, n.d).

ETS includes audits of member organizations to ensure adherence to the programs. The audit component of ETS and IRM is specific to the general accountability of the IRM program and records and documentation of the strategies and processes involved (Storer, n.d.). Companies perform internal audits to ensure compliance before external audits. The external audit is performed by ETS certified auditors and the audits are reported to ETD. The ETS audits certify organizations and provide an ETS in good standing membership status. This membership status is very important for agricultural companies due to trait licensing and agreements. A majority of agreements require an ETS in good standings membership status, and quality management system components for the use of competitor traits. ETS incorporates IRM with an integrated approach to management of pests.

IPM and IRM

Integrated Pest Management (IPM) has multiple definitions that all include the use of multiple strategies that benefit the environment, focus on pest prevention, and the use of pesticides only when it is necessary (Kogan, 1998). Excellence Through Stewardship includes IRM as a component of IPM and defines IPM as, “the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of resistant pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms.” (Excellence Through Stewardship, n.d). IPM has been noted throughout history and dates back to the 1800s. The term was not officially documented as IPM until 1972 (Kogan, 1998). IPM is essentially a long-term approach to pest management and involves the integration of several control methods in order to provide the most environmentally sound approach possible. A comprehensive understanding of the pest biology and behavior, inspection, monitoring, defined action thresholds, prevention techniques, and control methods are all components of IPM programs (United States Environmental, n.d.). IPM provides a safer and more environmentally friends approach to pest management by reducing the need for chemical control.

IPM integrates control methods based on several factors. These factors include inspection, monitoring, and reports (United States Environmental, n.d.). It is also important that the pest is identified correctly and there is determined and set action thresholds for the pest populations (United States Environmental, n.d.). The action thresholds range from a level of nuisance, health hazard, or economic threat and provide decision information for control methods (United States Environmental, n.d.). IPM also focus on pest prevention and using preventative measure to disrupt the favorable conditions for the pests. This may include removal of standing water, education of IPM, the use of pest barriers, and removing dense areas of foliage (United States Environmental, n.d.).

IPM and IRM together represent an integrated management perspective to provide long-term pest management and the IRM components should be incorporated into IPM. Monitoring for resistance and an integrated pest management approach is essential for success in long-term control. There are four factors of how IRM and IPM fit together. The four factors are multiple mechanisms of mortality, reduction in selection pressure, refuge to allow for mating of resistant and susceptible insects, and prediction methods using monitoring and models (Onstad, 2014). The EPA is moving towards the IPM approach as far as a proactive rather than reactive approach. This approach places an emphasis on resistance monitoring. Some of the IPM tools listed from the EPA include non-Bt corn, considering mode of action and alternating between them, and crop rotation (Reynolds, 2016).

V. Future Technologies for Control

With the increase in resistance to crop rotation, pesticides, and transgenic control, there is a need for enhanced pest management techniques. The future of pest management is evolving due to the immense advancements in technology. There are several new technologies breaking into the agriculture world that have the ability to edit genomes to increase plant defense against pests, increase the nutritional value, growth potential or yield potential, and the ability to thrive in less favorable conditions. These technologies also offer safer options for pest management that will hopefully lead to improved notions about biotechnology. For this paper, I am going to focus on RNA interference and CRISPR-Cas 9 (CRISPR) because these two technologies are most prevalent in the agricultural industry.

RNAi

RNA interference (RNAi) is a technology used for genetically engineering crops through gene silencing or gene blocking (Onstad, 2014). RNAi was first discovered in 2000 and has extreme potential due to the amount of genes available for silencing in insects (Onstad, 2014). The elements to focus on with RNAi are identification of an adequate insect molecular target, delivery of dsRNA, and specificity (Onstad, 2014). Studies with ingestion of dsRNA have proven very successful in both larvae and adult western corn rootworm. The two key components for success with western corn rootworm in regards to RNAi are uptake of dsRNA in the midgut and the spread of the RNAi signal throughout the body tissues of the insect (Fishilevich et al., 2016).

The gene silencing process begins with ingesting of double stranded RNA. The double stranded RNA is processed after delivery to the cells in to small interfering RNA. The small interfering RNA is then recognized by Argonaute proteins and this leads to the destruction of the mRNA (Fishilevich et al., 2016). Destruction of the mRNA and proteins essential for function of the cells ultimately leads to cell death. Using RNAi technology would decrease or eliminate the need for chemical control. The high degree of specificity would also help with non-target and beneficial organisms, and greatly benefit the environment. In regards to the IRM and EPA recommendations, RNAi would most likely be stacked with other Bt traits to deliver multiple modes of actions or involve a RNAi pyramid. There is a risk with releasing a single RNAi trait and development of resistance similar to what was experience with Bt. Another negative of RNAi is the slow speed of mortality and the lack of success with Lepidopteran species such as fall armyworm.

CRISPR

The CRISPR system Clustered Regularly Interspaced Short Palindromic Repeats, is a gene editing tool that is the new emerging technology in agriculture (Sanders, 2017). CRISPR was first discovered in a medical lab at UC Berkeley but has the potential for expansion of use in other industry areas. CRISPR has been recognized as a general global health gene editing tool (Sanders, 2017). The use of CRISPR technology in agriculture would allow for higher yields, offer draught and disease resistant varieties. The greatest benefit being that CRISPR functions without the addition of foreign DNA. CRISPR was initially discovered in 1987 in the *E. coli* genome but the function was not known until 2007 (Addgene, n.d.). CRISPR is naturally occurring segment of DNA involved in immune defense that is found in the genome of bacteria and other microorganisms.

The CRISPR-Cas9 system was developed in 2012 and is an easy to use and precise tool that involves two components. The two components are a guide RNA (gRNA) and Cas9, a non-specific CRISPR associated endonuclease (Addgene, n.d.). The Cas9 is a CRISPR protein is easily directed to a specific portion of DNA in the genome (Sanders, 2017). The protein then disables the specific gene which then allows for insertion of new DNA (Sanders, 2017). Cas are associated CRISPR genes that code for polymerases, nucleases and helicases (Addgene, n.d.). The Cas proteins are able to snip foreign DNA, add the fragments to the CRISPR sequence, and the CRIRPR RNAs are then expressed by other Cas proteins (Addgene, n.d.). CRISPR RNAs and a Cas nuclease are guided to the foreign material where the complex then attaches to the foreign DNA, slices it, and ultimately destroys it (Addgene, n.d.) CRISPR causes a permanent genome effect that is also heritable (Barrangou et al., 2015).

Advantages

The future technologies for control focus on the overall safety of the environment and human health. Both RNAi and CRISPR offer drastic benefits in the safety area when compared to other control methods of the past. There has been an increase in safety concerns with the use of genetically modified organisms and has led some to be scared of genetically modified ingredients in food altogether. CRISPR in particular does not involve any foreign DNA and could potentially provide some ease to those concerned about what they are putting in their bodies. As technology continues to advance, so will pest management strategies. The future of control should focus on new technologies and the integrated pest management approach.

VI. Conclusion

Pest management plays an important role in society due to the devastation that can occur to crops and ultimately our food and jobs. Western corn rootworm has been able to adapt and overcome multiple management strategies. The adaptation consistency really demonstrates how remarkable insects are and emphasizes the importance of understanding the general pest biology in order to advance in the area of pest management. The abundance of insect resistance has led to mandates through the Environmental Protection Agency and other government organizations that are attempting to adapt control measures to prevent or prolong resistance and extend trait durability

We have learned throughout history the importance or prevention rather than reacting with the quick fix and the previous disregard to the downstream effects. Advances in technology has allowed for developments of new management techniques that offer several advantages. The advantages not only include better pest management techniques but also benefit the environment and non-target populations. CRISPR and RNAi are examples of emerging technologies that have the potential to change the agricultural industry. These new technologies will hopefully eliminate the need for chemical control, decrease the development of resistance problems, and provide the best possible food to feed our world.

Society has become increasingly concerned with the environment and the safety of GMOs. It is important to consider all aspects from pest biology, prevention, IRM plans, human health and environmental safety. Integrated pest management provides a long-term approach to pest management and includes several strategies with a focus on prevention and the environment. The EPA has progressed the aspect of resistance in the industry by requiring contracts with growers, but it is important to educate the growers and the public about the level of detail and safety studies that are involved with any new product. From the beginning of pest management until now, there has been a significant amount of trial and error, quick successes, detrimental effects, and lessons learned. Insects are always evolving and it is important to improve on past mistakes, integrate control methods, and continue to advance technology in order to be successful in pest management.

Author's Statement

I have thoroughly enjoyed working on this project over the last 13 weeks. This project has allowed me to make connections between my career and the courses I have taken at the University of Nebraska-Lincoln. This project describes the history of pest management, western corn rootworm, and resistance management. Researching this paper allowed me to connect a lot of my knowledge and also fill the gaps of how everything relates between the agricultural companies, growers, and the EPA. The most interesting portion of this paper was learning about the EPA and the requirements. I was able to meet with resistance management employees at my company and gain a general understanding of how the EPA provides regulations and how they work with agricultural companies. This also helped me understand why specific questions are important to ask during IRM audits and how important these aspects are to and IRM program. The ETS component is a big part of my current role and we are very involved with ETS. I have personally conducted the internal audit of the ETS organization. I would like to thank all of my professors at the University of Nebraska-Lincoln and the Applied Science Program for allowing me to have a unique and diverse learning experience.

VII. Citation

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