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PERSPECTIVES ON INSECTICIDE RESISTANCE MANAGEMENT:
PAST, PRESENT, AND FUTURE

by

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PERSPECTIVES ON INSECTICIDE RESISTANCE MANAGEMENT:
PAST, PRESENT, AND FUTURE

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University of Nebraska, 2021

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The use of chemical products to control arthropod pests has occurred for thousands of years, beginning with the use of inorganic products in ancient cultures, and progressing to the development of synthetic insecticides beginning in the early 20th century. As these chemicals have imposed selection pressure on insects, the insects have adapted to this pressure, leading to the development of insecticide resistance. Since it was first reported in 1914, insecticide resistance has grown to be a major concern facing agricultural production, as each insecticide chemistry introduced is impacted by the evolution of resistance.

In the latter half of the 20th century, renewed focus was placed on non-chemical pest management practices and their use in Integrated Pest Management programs to help improve the sustainability of insect pest management and slow the development of insecticide resistance. New insect management products such as plants modified to express Plant Incorporated Protectants (PIPs) provided new options to producers who were interested in diversifying their management strategies, but they brought with them resistance management challenges of their own. To maintain the utility of insecticides for insect pest management, it is necessary to take steps to utilize effective Insecticide Resistance Management, through measures like rotating insecticide modes of action, utilizing resistant plants, planning planting and harvests to minimize pest pressure, and many others. These tactics will continue to be necessary in the future but increasing

emphasis on tactics such as areawide pest management and research into insect tolerant plant varieties will further help to reduce the pressure placed on insecticides in pest control. Research into the basis of resistance in insects will help inform decisions regarding resistance management approaches that may be effective. New technologies for PIPs such as RNAi can help to diversify the products available to producers and potentially help to increase the efficacy of tactics already in use. The future of Insecticide Resistance Management will require integrated, research-based solutions to maintain the sustainability of insect management.

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CHAPTER 1: THE HISTORY OF INSECTICIDE RESISTANCE

Insecticide resistance is an ever-growing concern in crop production. New insect species develop resistance to various chemistries every year, and some pest species have become very difficult to control due to their resistance to a wide variety of insecticides. This is not a new concern, however. Insecticide resistance has been observed in some species since shortly after the first synthetic pesticides were marketed, and it is likely these concerns will continue. To maintain the efficacy of our management practices and products, it will be important to continue to explore and discover new ways to manage insects effectively.

Humans have been utilizing insecticides in one form or another to protect crops from various insect pests for thousands of years. Ancient Sumerians and other cultures four thousand years ago utilized inorganic sulfur dusts to protect their crops (Oberemok et al., 2015). Other records from the same time mention using poisonous plants to protect against insect pests. Within the last several hundred years, several plant extracts with pesticidal properties have been identified and widely utilized for pest control. Dried dusts, known as “Persian dust,” produced from *Chrysanthemum roseum* and other closely related flowers which can be dried to produce an insecticidal dust known as pyrethrum (Casida, 1980), were utilized in Europe some 200 years ago for control of a variety of insect pests (Davies et al., 2007). Extracts of elderberry flowers, tobacco, and wormwood have all been shown to have insecticidal properties and have been used effectively against various insects (Oberemok et al., 2015).

In the middle of the 19th century, several new chemicals began to be used to protect plants from insects. The inorganic compound Paris green (copper-acetoarsenite) was first successfully applied in 1871 to aid in control of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), as it spread across the United States (Alyokhin, 2007). DDT was also first synthesized at around this time, though its insecticidal properties were not discovered until 1939 (Jarman & Ballschmiter, 2012). DDT was widely used during World War II for control of insect-borne disease in humans, though it later was also used for control of agriculturally relevant insects. It became widely used for control of codling moth (*Laspeyresia pomonella*), pink bollworm (*Pectinophora gossypiella*), and many other pests (Madsen & Hoyt, 1958; Tsao & Lowry, 1963). A number of other organochlorine insecticides, such as aldrin and dieldrin, were first utilized at around this same time. Within the next few decades, many of the synthetic insecticide families and chemistries that are widely used today were developed and released to the public. In the 1940s and 1950s, the first organophosphate insecticides were introduced, and the carbamates were introduced in the 1960s (Casida & Durkin, 2013).

At around this same time, the use of pyrethrins, the insecticidal esters providing the insecticidal properties of pyrethrum, reemerged in pest control, and research of their chemical structures led to the development of the first synthetic pyrethroid chemicals in the 1950s (Davies et al., 2007). Over the following decades, new, more stable pyrethroids were developed, and they entered widespread use in the 1970s (Matsuo, 2019). The relatively low mammalian toxicity of the pyrethroids greatly promoted the use of these chemicals over some of the other popular chemicals at the time.

The first neonicotinoid insecticide, imidacloprid, was commercialized in 1991, and it has since become one of the world's best-selling insecticides (Jeschke et al., 2011). The majority of the insecticide classes discussed thus far target different points in the insect nervous system. Other groups of insecticide chemistries have been discovered that target a wide variety of processes in insects. These include juvenile hormone mimics, which disrupt metamorphosis in immature insects, molting disruptors, and chemicals that disrupt cellular metabolism. The Insecticide Resistance Action Committee (IRAC) has classified insecticide chemistries that have 30 different modes of action against insects (IRAC International MoA Working Group, 2020). This classification scheme can be seen in Table 1.1.

While insecticides are a very useful tool in the pest management toolbox, they also carry with them risks, and improper management has led to widespread development of insecticide resistance. This chapter will explore the insecticide use trends of the 20th century, and how they led to the rapid development of insecticide resistance throughout the world, as well as some of the early attempts to slow this development and change the way insecticides were used. Chapter two will explore more recent developments and technological advancements related to insecticide resistance and its management, as well as discussing the extent of insecticide resistance and some of the most concerning cases of resistance. Finally, Chapter 3 will explore potential methods for the future to support insecticide resistance management, including alternative insect management methods and how new technologies can aid in understanding and managing insecticide resistance.

Table 1.1

Classes of insecticides in the IRAC mode of action classification scheme

IRAC Group	Class Name	Mode of Action ¹	Major AIs ²	Introduction ³	Resistance? ⁴
1A	Carbamates	AChE inhibitor	cabaryl carbosulfan	1950	Y
1B	Organophosphates	AChE inhibitor	chlorpyrifos malathion	1944	Y
2A	Cyclodiene Organochlorines	GGCC blocker	chlordane endosulfan	1950	Y
2B	Phenylpyrazoles	GGCC blocker	ethiprole fipronil	1990	Y
3A	Pyrethroids, Pyrethrins	Sodium Channel modulator	bifenthrin cyhalothrin	1977	Y
3B	DDT, Methoxychlor	Sodium Channel modulator	DDT methoxychlor	1944	Y
4A	Neonicotinoids	nAChR agonist	imidacloprid thiamethoxam	1990	Y
4B	Nicotine	nAChR agonist	nicotine	1763	N
4C	Sulfoximines	nAChR agonist	sulfoxaflor	2013	Y
4D	Butenolides	nAChR agonist	flupyradifurone	2014	Y
4E	Mesoionics	nAChR agonist	triflumezopyrim	-	N

(continued)

5	Spinosyns	nAChR modulator Site I	spinetoram spinosad	1997	Y
6	Avermectins	GLUCL modulator	abamectin	1978	Y
7A	Juvenile Hormone analogs	Juvenile Hormone mimic	hydroprene	1973	Y
7B	Fenoxycarb	Juvenile Hormone mimic	fenoxycarb	1985	Y
7C	Pyriproxyfen	Juvenile Hormone mimic	pyriproxyfen	1995	Y
8A	Alkyl Halides	Non-specific inhibitor	methyl bromide	1932	Y
8B	Chloropicrin	Non-specific inhibitor	chloropicrin	1908	Y
8C	Fluorides	Non-specific inhibitor	cryolite	2004	N
8D	Borates	Non-specific inhibitor	boric acid	-	N
8E	Tartar Emetic	Non-specific inhibitor	tartar emetic	-	N
8F	Methyl Isothiocyanate generators	Non-specific inhibitor	dazomet	-	N
9B	Pyridine Azomethine derivatives	Chordontal Organ TRPV modulator	pymetrozine	1994	Y
9D	Pyropenes	Chordontal Organ TRPV modulator	afidopyropen	-	N
10A	Clofentezine, Diflovidazin, Hexythiazox	Mite growth inhibitor	clofentezine	1983	Y
10B	Etoxazole	Mite growth inhibitor	etoxazole	1998	Y
11A	<i>Bacillus thuringiensis</i>	Midgut Membrane disruptor	<i>B.t.var. kurstaki</i>	1970	Y

(continued)

11B	<i>Bacillus sphaericus</i>	Midgut Membrane disruptor	<i>Bacillus sphaericus</i>	1982	Y
12A	Diafenthiuron	Mitochondrial ATP Synthase inhibitor	diafenthiuron	1991	Y
12B	Organotin Miticides	Mitochondrial ATP Synthase inhibitor	azocyclotin	1968	Y
12C	Propargite	Mitochondrial ATP Synthase inhibitor	propargite	1964	Y
12D	Tetradifon	Mitochondrial ATP Synthase inhibitor	tetradifon	1954	N
13	Pyroles, Dinitrophenols, Sulfuramid	Oxidative Phosphorylation uncoupler	chlorfenapyr	1892	Y
14	Nereistoxin analogs	nAChR channel blocker	bensultap	1965	Y
15	Benzoylureas	Chitin Biosynthesis inhibitor, CHS1	bistrifluron	1975	Y
16	Buprofezin	Chitin Biosynthesis inhibitor, Type 1	buprofezin	1984	Y
17	Cyromazine	Dipteran Molting disruptor	cyromazine	1985	Y
18	Diacylhydrazines	Ecdysone Receptor agonist	chromafenozide	1993	Y
19	Amitraz	Octopamine Receptor agonist	amitraz	-	Y
20A	Hydramethylnon	Mitochondrial Complex III inhibitor	hydramethylnon	1977	N
20B	Acequinocyl	Mitochondrial Complex III inhibitor	acequinocyl	1999	Y

(continued)

20C	Fluacrypyrim	Mitochondrial Complex III inhibitor	fluacrypyrim	2002	Y
20D	Bifenazate	Mitochondrial Complex III inhibitor	bifenazate	-	N
21A	METI Acaricides and Insecticides	Mitochondrial Complex I inhibitor	fenazaquin	1990	Y
21B	Rotenone	Mitochondrial Complex I inhibitor	rotenone	1848	Y
22A	Oxadiazines	Voltage-gated Sodium Channel blockers	indoxacarb	1997	Y
22B	Semicarbazones	Voltage-gated Sodium Channel blockers	metaflumizone	2007	Y
23	Tetronic and Tetramic acid derivatives	ACCase inhibitor	spirodiclofen	2002	Y
24A	Phosphides	Mitochondrial Complex IV inhibitor	aluminum phosphide	-	Y
24B	Cyanides	Mitochondrial Complex IV inhibitor	calcium cyanide	1877	N
25A	Beta-Ketonitrile derivatives	Mitochondrial Complex II inhibitor	cyenopyrafen	2007	Y
25B	Carboxanilides	Mitochondrial Complex II inhibitor	pyflubumide	-	N
28	Diamides	Ryanodine Receptor modulators	chlorantraniliprole	2008	Y
29	Flonicamid	Chordontal Organ modulator-undefined	flonicamid	-	Y
30	Meta-diamides, Isoxazolines	GGCC allosteric modulator	broflanilide	-	N

(continued)

31	Granuloviruses, Nucleopolyhedroviruses	Baculoviruses	<i>Cydia pomonella</i>	-	N
32	GS-omega/kappa HXTX- Hv1a peptide	nAChR modulator Site II	GS-omega/kappa HXTX-Hv1a peptide	-	N

¹ Abbreviations: AChE, acetylcholinesterase; GGCC, GABA gated chloride channel; nAChR, nicotinic acetylcholine receptor; GLUCL, glutamate-gated chloride channel; ACCase, acetyl CoA carboxylase

² Examples of active ingredients from each group. Based on data from <https://irac-online.org/modes-of-action/>

³ Approximate year of first introduction of compounds in the class. Adapted from Sparks and Nauen, 2015

⁴ Has resistance to insecticides in this class been reported? Based on data from Mota-Sanchez and Wise, 2021

1.1 Historical Development of Insecticide Resistance

Development of resistance to management techniques has long been a concern in entomology. The first documented case of resistance developing in the field was published in 1914, when an application of sulfur-lime at a rate 10 times stronger than the normal application rate left 74% of the targeted scale insects alive (Melander, 1914). Over the following 30 years, a variety of cases of resistance to several inorganic insecticidal compounds were reported in four insect orders: the Homoptera, Lepidoptera, Diptera, and Thysanoptera, and two species of Acari (Mota-Sanchez & Wise, 2021). After the release of the first synthetic chemistries, the organochlorines, the first reports of resistance were not far behind. DDT resistance was first observed in Italy in 1946, where houseflies began to reemerge in houses that had been treated for insect control (Brown & Pal, 1971). These authors also describe that in the same areas, over the next several years, mosquitoes targeted by these insecticide applications also began to develop resistance, and applications were no longer effective. At around the same time, bed bug resistance to DDT was reported in Hawaii (Johnson & Hill, 1948). The rapid speed at which this resistance began to develop was not an isolated incident. As new insecticide chemistries have continued to be released, at least some insect species from around the world have been shown to develop resistance to each of them within 2-20 years of their first use.

One insect species of note to the discussion of insecticide resistance is Colorado potato beetle. This species has become notable due to the extreme number of insecticide classes and modes of action to which it has evolved resistance. The first synthetic insecticide resistance reported for Colorado potato beetle was to DDT in 1952, and this was followed shortly by dieldrin and other organochlorine insecticides in the late 1950s.

Since this time, populations of potato beetles all over the world have been reported to be resistant to 52 insecticide active ingredients across almost all major insecticide classes (Alyokhin et al., 2008).

1.2 Factors in the Evolution of Insecticide Resistance

In order to slow resistance development today and potentially avoid such growth in the future, it is important to consider what trends in pest control may have driven the quick development and spread of insecticide resistance throughout the 20th century. Until the late 19th century, insect pest control utilized many cultural and physical control methods, rather than relying heavily on the few chemical controls available. Practices such as crop rotation, residue destruction, trap crops, and other similar methods were widely used, though they were not effective against all pests (Osteen & Szmedra, 1989). Then, with the development and initial success of Paris green, during the period of 1900-1965 many pest control scientists began to focus heavily on the development and use of chemical control and resistant plant varieties for insect pest management (Osteen & Szmedra, 1989). During this period, it became very common for agricultural producers to repeatedly apply the same pesticide in a field as scheduled prophylactic applications, regardless of the presence or absence of pests in the field (Furlong et al., 2013). This led to the persistent presence of residual pesticides in the field, helping to prevent insects from becoming established in the field in the first place. In addition to these repeated applications, these early insecticides often had very long residual persistence. This was particularly true of the organochlorine insecticides. For example, DDT has been shown to persist for years in the soil, with up to 55% of applied DDT remaining 15 years after the last application (Owen et al., 1977; Stewart & Chisholm, 1971). The primary

disadvantage of repeated insecticide applications was the repeated and often continuous selection pressure applied to the population for an insecticide, and only those individuals that are resistant within that population survive the application and are able to reproduce. This can lead to the rapid accumulation of resistant alleles in the population, resulting in the eventual breakdown of control by that pesticide.

Another important consideration with utilizing insecticides for pest control is the potential for exposure of certain members of the population to less-than lethal doses of the insecticide. This can result from incomplete coverage in an application, or the degradation of long-residual pesticides to sublethal doses (Guedes et al., 2017). This increases the survival chances of individuals that are resistant and individuals that are only partially resistant to the application. This can lead to more rapid accumulation of resistance alleles in the population, even if individuals possessing a mutation that provides high levels of resistance do not exist initially in the population (Bantz et al., 2011).

There is also the problem of non-target effects on other pest insects. When insecticides are sprayed on a schedule for control of a certain insect species, this fails to consider the potential presence of other pest insect species in the field. In some cases, these other pest species may have some natural resistance to the product being applied, which can be exacerbated by the selection pressure applied by these applications. Alternatively, they may have a different lifecycle or feeding habits that cause them to only be exposed to lower rates or small amounts of the insecticide. This can provide minor selection pressure that can lead to the evolution of resistance (Müller, 2018). Sublethal insecticide exposure can potentially improve the fitness of pest insects, through

an effect known as hormesis, in which small amounts of a toxin can stimulate growth (Margus et al., 2019).

Another potential non-target effect of greater reliance on insecticide applications is the loss of natural enemy species. These beneficial insects can be killed by insecticide applications, thereby eliminating a control option for the pest species (Regan et al., 2017). This can occur if the pest population is able to recover faster than the population of the natural enemies after an insecticide application, or if the natural enemies are more heavily impacted by the insecticide (Bommarco et al., 2011). The result of this can be resurgence of primary pest species when no natural controls are present following the application. Alternatively, insecticides killing natural enemies can result in replacement of the primary pest species with uncontrolled secondary pest species (Hill et al., 2017). In most plant-pest relationships, in addition to the primary damage-causing pest, there are also other pests present that are mostly controlled by the natural enemies present (Yang et al., 2016). When these natural enemies are killed or weakened by insecticide applications, it can release these secondary pests from natural enemy control, causing them to become much more damaging to the crop (Dutcher, 2007; Yang et al., 2016). A common example of this phenomenon can be seen in spider mites. Most insecticides that are used in agricultural production are more toxic to insect pests and natural enemies than the mites, and mite populations can grow quickly in the absence of natural enemies, allowing them to cause severe damage after some insecticide applications (Schmidt-Jeffris & Beers, 2018).

In the first part of the 20th century, up to approximately the mid-1960s, the focus within the agrochemical industry on researching the use and efficacy of insecticides

increased. This also led to a decrease in the focus that was placed on researching the ecological characteristics of pest insects and how they could be managed without chemical tactics (Osteen & Szmedra, 1989). This trend did change in the latter half of the 1960s and through the 1970s and 1980s, as some of the more negative impacts of insecticide applications became clearer, and Integrated Pest Management (IPM) gained more focus. Despite this, insecticides remained the primary management tactic for many pests. Insecticides were proving to be such an efficient method of controlling insects that other management strategies were not considered as necessary as they once had been. This thought process led to an overreliance on insecticides, eventually producing what some have called the “pesticide treadmill.” This term is credited to Robert van den Bosch, in his 1978 book *The Pesticide Conspiracy*. Van den Bosch was a strong proponent of biological control and IPM who was heavily opposed to widespread use of insecticides and the repeated replacement of one insecticide with another (Huffaker & Mackey, 1979). Insecticides were used to control pest insect damage to a crop, and as a result, the pests develop resistance to that chemical at that application rate. Rather than recognizing the resistance problem and working to contain it, the rate of application was often increased or the product used was changed to a new mode of action until the pests were once more controlled by the insecticide (Arbuckle, 2014). As a result, the insects then developed resistance to the new mode of action or rate, and the cycle continues, with the pests in question becoming ever more difficult to control as multiple types of resistance are accumulated in the population. While to an extent, this trend has continued in recent years, this has been somewhat offset by increased focus on IPM throughout the 1970s, 1980s, and 1990s. Beginning in 1972, the United States Department of

Agriculture has sponsored programs to support the use of IPM programs in a variety of crops, including cotton, citrus, and soybean, among others (Peshin & Dhawan, 2009).

The programs in the 1970s and 1980s were estimated to have contributed to a reduction of 70-80% in the use of more environmentally polluting insecticides (Peshin & Dhawan, 2009). However, it is possible that some of this reduction resulted from replacement of some products with others that are applied at lower rates.

An illustration of the pesticide treadmill and subsequent adoption of IPM can be seen in pear production in California. From the 1950s through the early 1970s, insecticides were the primary method of control for codling moth in California pears, but by the early 1970s, pest damage was increasing despite high use of insecticides, due to resistance development (Weddle et al., 2009). As a result, producers began exploring IPM practices, including pest monitoring. In the early 1990s, areawide management plans were initiated, reducing damage levels and observed populations of codling moth while also reducing the use of organophosphate insecticides by 96% over a 10 year period (Weddle et al., 2009).

The use of chemical products to protect crops from insect damage has been occurring for thousands of years. It is not until approximately the last 100 years, however, that we have realized that insects can often adapt to these products and overcome our control efforts. The development and overuse of synthetic insecticides led to a rapid buildup of resistance concerns throughout the 20th century. In the latter half of the century, efforts began to address these growing concerns and diversify management tactics in order to preserve the use of insecticides and the sustainability of agricultural

production. These efforts have not been entirely successful, and insecticide resistance remains a major concern in agriculture today.

References

- Alyokhin, A. (2007). *Colorado potato beetle management on potatoes: Current challenges and future prospects* *Potato Insect Pest Management View project*. Retrieved from <https://www.researchgate.net/publication/228617785>
- Alyokhin, A., Baker, M., Mota-Sanchez, D., Dively, G., & Grafius, E. (2008, December 24). Colorado potato beetle resistance to insecticides. *American Journal of Potato Research*. Springer. <https://doi.org/10.1007/s12230-008-9052-0>
- Arbuckle, J. G. (2014). Farmer Perspectives on Pesticide Resistance. *Extension Community and Economic Development Publications*, 25, 1–8. Retrieved from http://lib.dr.iastate.edu/extension_communities_pubs
- Bantz, A., Camon, J., Froger, J. A., Goven, D., & Raymond, V. (2018, December 1). Exposure to sublethal doses of insecticide and their effects on insects at cellular and physiological levels. *Current Opinion in Insect Science*. Elsevier Inc. <https://doi.org/10.1016/j.cois.2018.09.008>
- Bommarco, R., Miranda, F., Bylund, H., & Björkman, C. (2011). Insecticides Suppress Natural Enemies and Increase Pest Damage in Cabbage. *Journal of Economic Entomology*, 104(3), 782–791. <https://doi.org/10.1603/EC10444>
- Brown, A. W., & Pal, R. (1971). Insecticide resistance in arthropods. *Public Health Papers*, 38(0), 1–491.
- Casida, J. E. (1980). *Pyrethrum Flowers and Pyrethroid Insecticides*. *Environmental Health Perspectives* (Vol. 34).
- Casida, J. E., & Durkin, K. A. (2013). Anticholinesterase insecticide retrospective. *Chemico-Biological Interactions*, 203(1), 221–225. <https://doi.org/10.1016/j.cbi.2012.08.002>
- Davies, T. G. E., Field, L. M., Usherwood, P. N. R., & Williamson, M. S. (2007). DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB Life*, 59(3), 151–162. <https://doi.org/10.1080/15216540701352042>
- Dutcher, J. D. (2007). A review of resurgence and replacement causing pest outbreaks in IPM. In *General Concepts in Integrated Pest and Disease Management* (pp. 27–43). https://doi.org/10.1007/978-1-4020-6061-8_2
- Furlong, M. J., Wright, D. J., & Dosdall, L. M. (2013). Diamondback moth ecology and management: Problems, progress, and prospects. *Annual Review of Entomology*, 58(1), 517–541. <https://doi.org/10.1146/annurev-ento-120811-153605>
- Gressel, J. (2011). Low pesticide rates may hasten the evolution of resistance by

- increasing mutation frequencies. *Pest Management Science*, 67(3), 253–257.
<https://doi.org/10.1002/ps.2071>
- Guedes, R. N. C., Walse, S. S., & Throne, J. E. (2017, June 1). Sublethal exposure, insecticide resistance, and community stress. *Current Opinion in Insect Science*. Elsevier Inc. <https://doi.org/10.1016/j.cois.2017.04.010>
- Hill, M. P., Macfadyen, S., & Nash, M. A. (2017). Broad spectrum pesticide application alters natural enemy communities and may facilitate secondary pest outbreaks. *PeerJ*, 2017(12), e4179. <https://doi.org/10.7717/peerj.4179>
- Huffaker, C., & Mackey, N. (1979). Obituary: Robert van den Bosch. *Nature*, 279(5710), 272–273. <https://doi.org/10.1038/279272a0>
- IRAC International MoA Working Group. (2020). IRAC Mode of Action Classification Scheme. Retrieved February 16, 2021, from <https://irac-online.org/modes-of-action/>
- Jarman, W. M., & Ballschmiter, K. (2012, December 1). From coal to DDT: The history of the development of the pesticide DDT from synthetic dyes till Silent Spring. *Endeavour*. Elsevier Ltd. <https://doi.org/10.1016/j.endeavour.2012.10.003>
- Jeschke, P., Nauen, R., Schindler, M., & Elbert, A. (2011). Overview of the Status and Global Strategy for Neonicotinoids †. *J. Agric. Food Chem*, 59, 2897–2908. <https://doi.org/10.1021/jf101303g>
- Johnson, M. S., & Hill, A. J. (1948). Partial resistance of a strain of bed bugs to DDT residuals. *Med. News Letter*, 12, 26–28.
- Madsen, H. F., & Hoyt, S. C. (1958). Investigations with New Insecticides for Codling Moth Control. *Journal of Economic Entomology*, 51(4), 422–424. <https://doi.org/10.1093/jee/51.4.422>
- Margus, A., Piironen, S., Lehmann, P., Tikka, S., Karvanen, J., & Lindström, L. (2019). Sublethal Pyrethroid Insecticide Exposure Carries Positive Fitness Effects Over Generations in a Pest Insect. *Scientific Reports*, 9(1), 1–10. <https://doi.org/10.1038/s41598-019-47473-1>
- Matsuo, N. (2019). Discovery and development of pyrethroid insecticides. *Proceedings of the Japan Academy Series B: Physical and Biological Sciences*. Japan Academy. <https://doi.org/10.2183/pjab.95.027>
- Melander, A. L. (1914). Can Insects Become Resistant to Sprays? *Journal of Economic Entomology*, 7, 167–173.
- Mota-Sanchez, D., & Wise, J. C. (2021). The Arthropod Pesticide Resistance Database. Retrieved from <http://www.pesticideresistance.org>

- Müller, C. (2018, August 1). Impacts of sublethal insecticide exposure on insects — Facts and knowledge gaps. *Basic and Applied Ecology*. Elsevier GmbH. <https://doi.org/10.1016/j.baae.2018.05.001>
- Oberemok, V. V., Laikova, K. V., Gninenko, Y. I., Zaitsev, A. S., Nyadar, P. M., & Adeyemi, T. A. (2015, July 1). A short history of insecticides. *Journal of Plant Protection Research*. De Gruyter Open Ltd. <https://doi.org/10.1515/jppr-2015-0033>
- Osteen, C. D., & Szmedra, P. I. (1989). *Agricultural Pesticide Use Trends and Policy Issues*. Resources and Technology Division, Economic Research Service, U.S. Department of Agriculture. Agricultural Economic Report No. 622. <https://naldc.nal.usda.gov/download/CAT10407750/PDF>
- Owen, R. B., Dimond, J. B., & Getchell, A. S. (1977). DDT: Persistence in Northern Spodosols. *Journal of Environmental Quality*, 6(4), 359–360. <https://doi.org/10.2134/jeq1977.00472425000600040005x>
- Peshin, R., & Zhang, W. (2009). Integrated Pest Management: A Global Overview of History, Programs and Adoption. In R. Peshin & A. K. Dhawan (Eds.), *Integrated Pest Management (Vol. 1)* (pp. 1-49). <https://doi.org/10.1007/978-1-4020-8992-3>
- Regan, K., Ordosch, D., Glover, K. D., Tilmon, K. J., & Szczepaniec, A. (2017). Effects of a pyrethroid and two neonicotinoid insecticides on population dynamics of key pests of soybean and abundance of their natural enemies. *Crop Protection*, 98, 24–32. <https://doi.org/10.1016/j.cropro.2017.03.004>
- Schmidt-Jeffris, R. A., & Beers, E. H. (2018). Potential impacts of orchard pesticides on *Tetranychus urticae*: A predator-prey perspective. *Crop Protection*, 103, 56–64. <https://doi.org/10.1016/j.cropro.2017.09.009>
- Sparks, T. C., & Nauen, R. (2015). IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*, 121, 122–128. <https://doi.org/10.1016/j.pestbp.2014.11.014>
- Stewart, D. K. R., & Chisholm, D. (1971). Long-term persistence of BHC, DDT and chlordane in a sandy loam soil. *Canadian Journal of Soil Science*, 51(3), 379–383. <https://doi.org/10.4141/cjss71-051>
- Tsao, C. H., & Lowry, W. L. (1963). Effect of DDT on Pink Bollworm Populations. *Journal of Economic Entomology*, 56(3), 388–390. <https://doi.org/10.1093/jee/56.3.388>
- Weddle, P. W., Welter, S. C., & Thomson, D. (2009). History of IPM in California pears - 50 years of pesticide use and the transition to biologically intensive IPM. *Pest Management Science*, 65(12), 1287–1292. <https://doi.org/10.1002/ps.1865>

Yang, L., Elbakidze, L., Marsh, T., & McIntosh, C. (2016). Primary and secondary pest management in agriculture: balancing pesticides and natural enemies in potato production. *Agricultural Economics*, 47(6), 609–619.
<https://doi.org/10.1111/agec.12259>

CHAPTER 2: THE CURRENT STATUS OF INSECTICIDE RESISTANCE AND RESISTANCE MANAGEMENT

Insecticide resistance is a concern that has emerged and magnified greatly over approximately the last century, with the number of reported cases of insecticide resistance growing exponentially. Currently, resistance has been reported to 349 different insecticide and acaricide active ingredients across 612 species all around the world, for a total of over 17,000 reported cases of insecticide resistance (Mota-Sanchez & Wise, 2021). Even with this concern, however, insecticides remain a crucial tool in the insect pest management toolbox, though the ways in which they are used today differs from the past. Over the last few decades, the amount of insecticide used in crop protection has decreased. According to data collected in USDA pesticide use surveys, across 21 selected crops, 105 million pounds of insecticide active ingredient were used in 1980, while this number had decreased to 28 million pounds in 2008 (Fernandez-Cornejo et al., 2011). This decline in insecticide use has occurred due to several factors. One of these factors is increased implementation and utilization of Integrated Pest Management (IPM) programs.

While there are many insecticides and insect species where some level of resistance has been reported, there are a smaller number of species where resistance has become an especially major concern. These include both agricultural pest species and human disease vectors. Sparks and Nauen (2015) provide a list of the 12 most resistant arthropod species at the time: Two-spotted spider mite, *Tetranychus urticae*, was resistant to 93 compounds; diamondback moth, *Plutella xylostella*, was resistant to 91 compounds;

green peach aphid, *Myzus persicae*, was resistant to 75 compounds; house fly, *Musca domestica*, was resistant to 58 compounds. The next species on Sparks and Nauen's list is one of significant agricultural concern: silverleaf whitefly, *Bemisia tabaci*. Whitefly is one of the top agricultural pests globally, and Sparks and Nauen reported it to have resistance to 54 compounds in 2015, though this number has grown to more than 60 insecticide active ingredients, including many from major insecticide classes such as organophosphates, carbamates, pyrethroids, and neonicotinoids, among others (Horowitz et al., 2020). Another agricultural pest of concern is the Colorado potato beetle, *Leptinotarsa decemlineata*. This species has been a major pest of concern in potato production across the United States, and as a result, attempts have been made to control it with a wide variety of insecticide chemistries. In response to this, populations of potato beetle have developed resistance to almost all commonly used insecticide active ingredients and modes of action, and resistance had been reported to 52 different insecticide active ingredients by 2008 (Alyokhin et al., 2008). This includes carbamates, organophosphates, pyrethroids, organochlorines, and neonicotinoids, as well as several smaller insecticide classes. Sparks and Nauen (2015) report that this number had grown to 54 active ingredients. There are a number of other relevant pests on Sparks and Nauen's list, including cotton aphid (*Aphis gossypii*), European red mite (*Panonychus ulmi*), cotton bollworm (*Helicoverpa armigera*), southern cattle tick (*Boophilus microplus*), German cockroach (*Blattella germanica*), and Mediterranean climbing cutworm (*Spodoptera litura*). The number of compounds for which resistance had been reported in these species ranged from 38 to 48. From a human health perspective, the most prominent cause for concern in insecticide resistance is in mosquito species in the

genera *Aedes* and *Anopheles*. These species are vectors of a number of human viruses, as well as malaria, and resistance has been observed to a variety of insecticide chemistries, including carbamates, organochlorines, organophosphates, and pyrethroids (Camara et al., 2018; Moyes et al., 2017).

Through the years the western corn rootworm (*Diabrotica virgifera virgifera*) has been a major insecticide resistance issue in the Midwest corn belt. This species has developed resistance to a wide variety of insecticidal chemicals that have been utilized in its control. The first documented case of insecticide resistance in rootworms comes from Nebraska in 1959, when reduced efficacy of aldrin and heptachlor was observed (Ball & Weekman, 1962). These organochlorine insecticides were applied to the soil in broadcast applications, and they are the only documented cases of soil-applied insecticide resistance in *D. virgifera*. There are two major factors that are thought to have contributed to this resistance developing. The first is the long soil half-life of these chemicals, leading to constant exposure and selection pressure for these chemicals in the insects (Lichtenstein et al., 1960). In addition, the organochlorine insecticides were applied to the soil in broadcast applications, differently than later soil-applied insecticides, which are applied either in-furrow or as a band over the row, resulting in a built-in refuge for susceptible individuals in the areas between the rows, slowing the spread of resistance alleles within the population (Meinke et al., 2021). As a result of this built-in refuge, the vast majority of insecticide resistance cases that have been observed in corn rootworm have resulted from the broadcast foliar application of those insecticides to control adult rootworm beetles later in the growing season.

2.1 The Insecticide Resistance Action Committee and Resistance Management

In 1984, a group of representatives from leading agrochemical companies formed a working group known as the Insecticide Resistance Action Committee (IRAC) to identify resistance and work toward developing resistance management strategies. Today, the goals of IRAC include facilitating communication and education concerning insecticide resistance and promoting development of resistance management strategies to maintain insecticide efficacy (Sparks & Nauen, 2015). IRAC has developed a comprehensive classification of insecticide modes of action, grouping insecticide active ingredients by the physiological function that they affect. Insecticides are classified using a number and letter, the number representing the physiological target (i.e. mode of action) of the insecticide, and the letter representing the insecticide family to which the active ingredient belongs. For example, carbamate and organophosphate insecticides are both classified as Acetylcholinesterase inhibitors and are designated as groups 1A and 1B, respectively. This classification indicates that both of these families have the same physiological target, and therefore should not be used successively for insect control. The complete IRAC mode of action classification can be found online at <https://irac-online.org/modes-of-action>. IRAC also provides summaries of insecticide resistance reports and issues recommendations for insecticide resistance management.

2.2 Insecticide Resistance Management

In the last 40-50 years, insecticide resistance management (IRM) has become an integral part of pest management and entomological research. If we hope to maintain the ability to use insecticides to control insect pests, it is necessary to take steps now to slow and prevent the development and spread of insecticide resistance through a variety of

tactics. All of the available options for insect management should be considered when creating management plans. Making a plan for the season and utilizing crop varieties that may mature early or are resistant to insect damage can help to prevent unnecessary insecticide applications (IRAC, 2013). Utilizing integrated management tactics and scouting fields to identify the most effective timing of applications can also help to reduce insecticide use and resulting selection pressure. A crucial aspect of this process is proper rotation of insecticides that are used. The fastest driver of insecticide resistance development is repeated application of the same insecticide or mode of action. Each time the insecticide is applied, most of the individuals in the pest population that are susceptible to the product are killed, while the individuals that have genetic mutations that allow them to resist the application are able to survive. This means that a greater proportion of the insects that are able to reproduce have these resistance alleles. As a result, the following generation contains a greater proportion of individuals that possess resistant alleles (Food and Agriculture Organization, 2012). If the same insecticide is applied again to this new generation, once again a greater proportion of susceptible individuals are killed, while resistant individuals survive, once again increasing the frequency of the resistance alleles in the population. After repeated generations of this type of selection, resistance eventually builds to a high enough level in the field that the insecticide is no longer effective to control the pest (South & Hastings, 2018). This process has also been demonstrated under laboratory conditions (Brown & Payne, 1988; Feng & Isman, 1995). When rotation is used, the selection pressure for the original insecticide is reduced as selection is imposed for a different mode of action each season or each generation, slowing the accumulation of resistant alleles.

Mixtures of chemistries have also been hypothesized to provide the same benefit as rotation because when selection is applied by several modes of action concurrently, it is much more difficult for the insects to develop resistance (Cloyd, 2010). In spite of this, they are not typically recommended for insecticide resistance management, as they pose a risk of the insects developing double resistance, and they must be well regulated to be effective (Tabashnik, 1989). The position of IRAC concerning insecticide mixtures is that they should primarily be utilized for pest management rather than resistance management. Insecticide mixtures can improve control of a pest or control a broader spectrum of pests by utilizing multiple modes of action (IRAC, 2013). There are several factors that should be considered if a mixture is being used for IRM. These include: individual products in the mixture should all be highly effective against the target pest, components of mixtures should not have the same mode of action classification, known cross-resistance between mixture components must be considered, mixtures are less effective if resistance is developing to one or both active ingredients, and both components should have similar residual activity periods to maximize IRM benefits (The IRAC Executive, 2012).

Insecticide resistance management is a crucial aspect of IPM, as it plays a role in maintaining the utility of insecticide use, which is a tactic of IPM. At the same time, proper use of IPM plays a role in managing insecticide resistance. The term IPM was first used in the early 1970s, and it focused on making the management of insect pests a more holistic process. Many of the tactics used in IPM, such as cultural practices, biological controls, and mechanical practices, were utilized long before the term was first used, but they became less common through the early 20th Century as insecticides became more

effective and research focused on their development (Osteen & Szmedra, 1989). The refocusing of the insect management community on the importance of properly utilizing integrated control tactics caused a shift in the use of insecticidal products. One aspect of this shift is the encouragement of proper scouting and decision-making for insect control based on the economic justification of utilizing a control tactic. Rather than spraying a field on a set schedule to prevent any insect damage at all from occurring, this tactic promotes evaluating the economic value of the damage that an insect can cause against the cost of implementing a management strategy to determine if management is economically justified. This also relates to one focus of IPM, that of managing plant damage rather than controlling insects (Peterson et al., 2018).

2.3 Bt crops as an IRM Tool with its own Resistance Challenges

The development and use of insect-resistant GM crop varieties has also contributed to the decline in chemical insecticide use (Brookes & Barfoot, 2013; Fitt, 2008). These consist of crop hybrids and varieties that have been genetically modified to express insecticidal proteins, sometimes referred to as Plant-incorporated Protectants, or PIPs. The most commonly used PIPs rely on Cry proteins produced by *Bacillus thuringiensis*. These proteins provide control of insect pests in addition to or in place of chemical insecticide applications, thereby helping to reduce selection pressure for insecticide resistance development (Nelson & Alves, 2013). In the ten year period from 1996-2006, the use of insect resistant corn and cotton led to an annual decrease of approximately 101.5 million kilograms in the amount of insecticide active ingredients used globally (Brookes & Barfoot, 2006).

The development of these varieties has occurred within the last few decades. The first commercialization of Bt crop varieties occurred in 1995 when Monsanto released potatoes expressing the Cry3A toxin to target the Colorado potato beetle. These potatoes were not widely adopted, and were removed from the market in 2001 (Thornton, 2004). Also in 1995, Syngenta and Mycogen released corn hybrids expressing a Cry1Ab gene, which was marketed for use against European corn borer, *Ostrinia nubilalis* (Sanchis, 2011). These early Bt varieties were quickly removed from the market, however, in favor of new events. In the following years, crop varieties expressing a number of new Cry proteins were developed and approved by the EPA. These included Cry1Ab1 in corn and Cry1Ac in cotton in 1995, Cry1Ac in tomatoes in 1998, Cry1F in corn in 2001. All of these varieties were targeted against lepidopteran pests. In 2002, the first plant variety containing two “pyramided” traits that target the same pest was released: cotton that expressed Cry1Ac and Cry2Ab, both targeting lepidopteran cotton pests (Sanchis, 2011). In 2003 came the first variety with “stacked” traits, which target different pest species. This was a corn variety from Monsanto which expressed Cry1Ab1 to target lepidopteran pests and Cry3Bb1 to target corn rootworm (Sanchis, 2011). The development and implementation of Bt crops continues in the present day.

While Bt-expressing crops serve an important purpose as part of IRM strategies, they also present a resistance concern of their own. Just as with chemical control tactics, pests can adapt to the Bt proteins they are challenged with and develop resistance to them. This eventuality was considered with the first introductions of Bt-expressing crops. As a result, when these crops were commercialized, the companies releasing them released an associated resistance management plan to delay the development of resistance

to these crops (Head & Greenplate, 2012). The majority of these plans utilized the “high dose-refuge” strategy. This strategy states that Bt crops should express the protein at a high enough dosage to kill greater than 99% of insects that are heterozygous for resistance (Gould, 1998). This ensures that only homozygous resistant individuals survive the toxin. When high-dose plants are used in conjunction with a refuge of a non-traited (susceptible) variety, these resistant individuals can mate with susceptible individuals, maintaining susceptible alleles in the population. The resulting heterozygous offspring can then be killed by the high-dose plants, preventing them from passing on resistance alleles (Huang et al., 2011). Two forms of refuge have been utilized. The first is a structured refuge, in which a block of susceptible plants is planted in or near the field. The initial requirements stated that refuge size must be equivalent to 20% of the field size for most Bt traits (Reisig, 2017). The second type of refuge is a seed mixture, in which susceptible seed is mixed with traited seed at a rate of 5-10% (Carroll et al., 2012). Both of these refuge types have advantages and disadvantages. The primary disadvantage of structured refuges are the requirement of the producer to separately plant the refuge area, which led to incomplete compliance with the requirements (Reisig, 2017), as compared to a mixed refuge, which is planted as a part of the normal planting of the field. On the other hand, the movement of larvae among plants could reduce the effectiveness of mixed refuges, if larvae move between refuge plants and nearby traited plants, thereby limiting exposure to the toxin (Carroll et al., 2012). This is opposed to the structured refuge, in which larval movement is less of a concern, due to the lack of proximity between individual susceptible and resistant plants. Structured refuges also rely on the movement of individual insects to find mates, which is common in species like the European corn

borer (*Ostrinia nubilalis*). Corn borer adults can disperse up to a half mile before mating, meaning that there is a high chance of random mating between susceptible and resistant individuals (Siegfried & Hellmich, 2012). As a result, structured refuges have been successful in delaying the development of resistance in corn borers.

Despite resistance management measures, insect resistance to Bt crops was developed, with the first reported species with resistance being cotton bollworm (*Helicoverpa zea*) in 2002 (Tabashnik et al., 2013). One suspected reason for the failure of the high-dose refuge strategy is lack of compliance with refuge requirements by producers. It is likely that many producers were unwilling or unable to comply with refuge requirements. A survey conducted in 2001 indicated that while 90% of the farmers surveyed indicated that they followed refuge requirements, only 71% of them were able to accurately define the required size and locations of refuges (Dove, 2001). As a result, it is likely that refuges were not always properly implemented, thereby weakening efforts to prevent the development of resistance. Another potential failure of resistance management resulted from the failure to develop high-dose plant varieties. This is especially prominent in varieties of corn expressing proteins targeted for western corn rootworm. None of the Bt corn varieties that have been released and utilized for control of western corn rootworm truly meet the high-dose requirement (Devos et al., 2013). They are instead considered to have a low-to-moderate expression of Bt toxins after further research. As a result, particularly in corn rootworm, much of the evolution of Bt resistance could be a result of the failure of Bt hybrids to cause the necessary levels of mortality, resulting in the survival of heterozygous individuals, which helped to increase the frequency of resistance alleles in populations (Gassmann, 2021).

2.4 IRM for Tropical Agriculture Pests

Insecticide resistance in tropical and subtropical insect species is a significant problem facing agriculture in these areas. In this type of climate, agricultural producers are able to grow crops throughout the year, without the off-season that occurs in more temperate climates during the colder months. This means that it is possible, and in many of these areas, rather common, for a crop species to be actively growing in an area year-round. As a result, insect species that feed on these crops are provided with a constant food source. Crop rotation at the individual field level is not an effective method of insect control in these areas because it is likely that as one field is harvested, another nearby field could have the same crop recently planted and newly growing. As a result of these factors among others, a number of lepidopteran species have become significant pests in corn, cotton, and soybean in Brazil. These species include the cotton bollworm (*Helicoverpa armigera*), the corn earworm (*Helicoverpa zea*), and the tobacco budworm (*Heliothis virescens*). The cotton bollworm was reported to have caused around \$800 million in damages during the 2012-2013 crop season in Brazil (Bueno & Sosa-Gómez, 2014). These species all have relatively broad host ranges and attack a variety of field crops. This is why it is especially troubling to see them developing resistance to a variety of insecticide chemistries. All three species have shown resistance to organophosphates, carbamates, pyrethroids, and *Bacillus thuringiensis* Cry proteins. A number of steps are being taken in these areas to implement IRM tactics, such as developing Economic Injury Level estimates for insect scouting to make efficient management decisions, implementing proper refuges for Bt crop varieties, and using burndown herbicide

applications to control weeds and volunteer plants that can serve as alternate hosts for the pests prior to planting new crops (Bortolotto et al., 2015; Pomari-Fernandes et al., 2015).

Resistance to insecticides is a major concern in today's agriculture. Over 600 arthropod species have developed resistance to over 300 insecticide active ingredients, straining the ability of producers to protect their crops from damaging pests. Over the last several decades, however, scientists and producers have taken the lessons learned during the first half of the 20th century regarding insecticide resistance development. They have worked to improve our usage of these products by developing IPM systems that allow for reduced insecticide use without compromising productivity. The establishment of groups like IRAC has helped to consolidate and distribute information and recommendations regarding proper insecticide use and IRM practices. As a result, insecticide use has decreased, and the development of resistance has slowed. New technologies like Bt crops have allowed producers to effectively control some insect pests, but these crops have also brought resistance challenges of their own. Trends in the use of insecticides for pest control seem to be moving in the right direction, but there is still work to be done, and new technologies and ways of thinking will help to continue improving the sustainability of insect control in agriculture.

References

- Alyokhin, A., Baker, M., Mota-Sanchez, D., Dively, G., & Grafius, E. (2008, December 24). Colorado potato beetle resistance to insecticides. *American Journal of Potato Research*. Springer. <https://doi.org/10.1007/s12230-008-9052-0>
- Ball, H. J., & Weekman, G. T. (1962). Insecticide Resistance in the Adult Western Corn Rootworm in Nebraska. *Journal of Economic Entomology*, 55(4), 439–441. <https://doi.org/10.1093/jee/55.4.439>
- Bortolotto, O. C., Pomari-Fernandes, A., De, R. C. O., Bueno, F., De, A., Da Kruz, Y. K. S., ... Ferreira, R. B. (2015). *The use of soybean integrated pest management in Brazil: a review. Agronomy Science and Biotechnology* (Vol. 1). Retrieved from www.asbjournal.com Freelyavailableonline
- Brookes, G., & Barfoot, P. (2006). *GM Crops: The First Ten Years - Global SocioEconomic and Environmental Impacts* (Vol. 36). Retrieved from <http://croplife.intraspain.com/Biotech/gm-crops-the-first-ten-years-global-socio-economic-and-environmental-impacts-isaaa-briefing-no-36/>
- Brookes, G., & Barfoot, P. (2013). The global income and production effects of genetically modified (GM) crops 1996-2011. *GM Crops & Food*, 4(1), 74–83. <https://doi.org/10.4161/gmcr.24176>
- Brown, T. M., & Payne, G. T. (1988). Experimental Selection for Insecticide Resistance. *Journal of Economic Entomology*, 81(1), 49–56. <https://doi.org/10.1093/jee/81.1.49>
- Bueno, A. de F., & Sosa-Gómez, D. R. (2014). The old world bollworm in the neotropical region: The experience of Brazilian growers with *Helicoverpa Armigera*. *Outlooks on Pest Management*, 25(4), 261–264. https://doi.org/10.1564/v25_aug_04
- Camara, S., Koffi, A. A., Ahoua Alou, L. P., Koffi, K., Kabran, J. P. K., Koné, A., ... Pennetier, C. (2018). Mapping insecticide resistance in *Anopheles gambiae* (s.l.) from Côte d'Ivoire. *Parasites and Vectors*, 11(1), 1–11. <https://doi.org/10.1186/s13071-017-2546-1>
- Carroll, M. W., Head, G., & Caprio, M. (2012). When and where a seed mix refuge makes sense for managing insect resistance to Bt plants. *Crop Protection*, 38, 74–79. <https://doi.org/10.1016/j.cropro.2012.02.015>
- Cloyd, R. A. (2010). Pesticide Mixtures and Rotations: Are these Viable Resistance Mitigating Strategies? *Pest Technology*, 4(1), 14–18. Retrieved from [http://www.globalsciencebooks.info/Online/GSBOnline/images/2010/PT_4\(1\)/PT_4\(1\)14-18o.pdf](http://www.globalsciencebooks.info/Online/GSBOnline/images/2010/PT_4(1)/PT_4(1)14-18o.pdf)
- Devos, Y., Meihls, L. N., Kiss, J., Hibbard, B. E. (2013). Resistance evolution to the first

generation of genetically modified Diabrotica-active Bt-maize events by western corn rootworm: management and monitoring considerations. *Transgenic Res*, 22, 269–299. <https://doi.org/10.1007/s11248-012-9657-4>

- Dove, A. (2001). Survey raises concerns about Bt resistance management. *Nature Biotechnology*, 19(4), 293–294. <https://doi.org/10.1038/86623>
- Feng, R., & Isman, M. B. (1995). Selection for resistance to azadirachtin in the green peach aphid, *Myzus persicae*. *Experientia*, 51(8), 831–833. <https://doi.org/10.1007/BF01922438>
- Fernandez-Cornejo, J., Nehring, R., Osteen, C., Wechsler, S., Martin, A., & Vialou, A. (2011). Pesticide use in U.S. agriculture: 21 selected crops, 1960-2008. *Agricultural Pesticides: Usage Trends and Analysis of Data Sources*, 1–102. <https://doi.org/10.2139/ssrn.2502986>
- Fitt, G. P. (2008). Have Bt Crops Led to Changes in Insecticide Use Patterns and Impacted IPM? In *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs* (pp. 303–328). Springer Netherlands. https://doi.org/10.1007/978-1-4020-8373-0_11
- Food and Agriculture Organization. (2012). *International Code of Conduct on the Distribution and Use of Pesticides: Guidelines on Prevention and Management of Pesticide Resistance. Assessment*. Retrieved from http://www.who.int/whopes/resources/resources_2010/en/
- Gassmann, A. J. (2021). Resistance to Bt Maize by Western Corn Rootworm: Effects of Pest Biology, the Pest–Crop Interaction and the Agricultural Landscape on Resistance. *Insects*, 12(2), 136. <https://doi.org/10.3390/insects12020136>
- Gould, F. (1998). Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology. *Annual Review of Entomology*, 43(1), 701-726. <https://doi.org/10.1146/annurev.ento.43.1.701>
- Head, G. P., & Greenplate, J. (2012). The design and implementation of insect resistance management programs for Bt crops. *GM Crops & Food*, 3(3), 144–153. <https://doi.org/10.4161/gmcr.20743>
- Horowitz, A. R., Ghanim, M., Roditakis, E., Nauen, R., & Ishaaya, I. (2020). Insecticide resistance and its management in *Bemisia tabaci* species. *Journal of Pest Science*. Springer Berlin Heidelberg. <https://doi.org/10.1007/s10340-020-01210-0>
- Huang, F., Andow, D. A., & Buschman, L. L. (2011, July 1). Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America. *Entomologia Experimentalis et Applicata*. John Wiley & Sons, Ltd. <https://doi.org/10.1111/j.1570-7458.2011.01138.x>

- IRAC. (2013). *General Principles of Insecticide Resistance Management from IRAC*. Retrieved from <http://www.iraconline.org>
- Lichtenstein, E. P., DePew, L. J., Eshbaugh, E. L., & Slesman, J. P. (1960). Persistence of DDT, Aldrin, and Lindane in Some Midwestern Soils. *Journal of Economic Entomology*, *53*(1), 136–142. <https://doi.org/10.1093/jee/53.1.136>
- Meinke, L. J., Souza, D., & Siegfried, B. D. (2021). The use of insecticides to manage the western corn rootworm, *diabrotica virgifera virgifera*, leconte: History, field-evolved resistance, and associated mechanisms. *Insects*, *12*(2), 1–22. <https://doi.org/10.3390/insects12020112>
- Mota-Sanchez, D., & Wise, J. C. (2021). The Arthropod Pesticide Resistance Database. Retrieved from <http://www.pesticideresistance.org>
- Moyes, C. L., Vontas, J., Martins, A. J., Ng, L. C., Koou, S. Y., Dusfour, I., ... Weetman, D. (2017). Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLOS Neglected Tropical Diseases*, *11*(7), e0005625. <https://doi.org/10.1371/journal.pntd.0005625>
- Nelson, M. E., & Alves, A. P. (2013). *Plant Incorporated Protectants and Insect Resistance. Insect Resistance Management: Second Edition*. Elsevier Ltd. <https://doi.org/10.1016/B978-0-12-396955-2.00004-7>
- Osteen, C. D., & Szmedra, P. I. (1989). *Agricultural Pesticide Use Trends and Policy Issues*. Resources and Technology Division, Economic Research Service, U.S. Department of Agriculture. Agricultural Economic Report No. 622. <https://naldc.nal.usda.gov/download/CAT10407750/PDF>
- Peterson, R. K. D., Higley, L. G., & Pedigo, L. P. (2018). Whatever Happened to IPM? *American Entomologist*, *64*(3), 146–150. <https://doi.org/10.1093/ae/tmy049>
- Pomari-Fernandes, A., De Freitas Bueno, A., & Sosa-Gómez, D. R. (2015). *Helicoverpa armigera*: current status and future perspectives in Brazil. *Current Agricultural Science and Technology*, *21*(1), 1–7. <https://doi.org/10.18539/cast.v21i1.4234>
- Reisig, D. D. (2017). Factors Associated With Willingness to Plant Non-Bt Maize Refuge and Suggestions for Increasing Refuge Compliance. *Journal of Integrated Pest Management*, *8*(1), 9. <https://doi.org/10.1093/jipm/pmx002>
- Sanchis, V. (2011, January 1). From microbial sprays to insect-resistant transgenic plants: History of the biopesticide *Bacillus thuringiensis*. A review. *Agronomy for Sustainable Development*. Springer. <https://doi.org/10.1051/agro/2010027>
- Siegfried, B. D., & Hellmich, R. L. (2012). Understanding successful resistance management: the European corn borer and Bt corn in the United States. *GM Crops*

& *Food*, 3(3), 184-193. <https://doi.org/10.4161/gmcr.20715>

- South, A., & Hastings, I. M. (2018). Insecticide resistance evolution with mixtures and sequences: A model-based explanation. *Malaria Journal*, 17(1), 80. <https://doi.org/10.1186/s12936-018-2203-y>
- Sparks, T. C., & Nauen, R. (2015). IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*, 121, 122–128. <https://doi.org/10.1016/j.pestbp.2014.11.014>
- Tabashnik, B. E. (1989). *Managing Resistance with Multiple Pesticide Tactics: Theory, Evidence, and Recommendations*. *J. Econ. Entomol* (Vol. 82). Retrieved from <https://academic.oup.com/jee/article/82/5/1263/2215220>
- Tabashnik, B. E., Brévault, T., & Carrière, Y. (2013, June). Insect resistance to Bt crops: Lessons from the first billion acres. *Nature Biotechnology*. <https://doi.org/10.1038/nbt.2597>
- The IRAC Executive. (2012). *IRAC International Insecticide Mixture Statement*. Retrieved from www.irc-online.org
- Thornton, M. (2004). The Rise and Fall of NewLeaf Potatoes. *NABC Rep.*, 15. http://nabc.cals.cornell.edu/Publications/Reports/nabc_15/15_7_2_Thornton.pdf

CHAPTER 3: INSECTICIDE RESISTANCE MANAGEMENT STRATEGIES FOR THE FUTURE

Insecticide resistance is a major concern in modern day agriculture, and those concerns will likely continue into the future. This is due to the fact that the use of insecticides remains an important aspect of managing insect pests. New research is exploring the increased reliance on Integrated Pest Management (IPM) for insect management, but it is not likely that the use of insecticides will be able to be eliminated. This is due to the fact that insecticide use can be an important facet of IPM programs. New insecticide modes of action and insect management techniques are constantly in development, but this will not help in the long term if we do not modify the way we use insecticides to slow the development of resistance. Simply introducing new insecticides without considering how to manage resistance development will only result in insects developing resistance to the new modes of action. Reliance on continuing the pesticide treadmill has another potential downside as well. The rate at which new modes of action are able to be developed and commercialized is slowing, due to increasing costs. It is becoming ever more labor and capital intensive to perform the required research to discover and commercialize new chemistries. The cost of discovery and development for a new pesticide increased from approximately \$4 million in 1960 (\$31 million adjusted for inflation) to over \$250 million in 2012 (Sparks, 2013). This price can likely be expected to continue to increase, meaning that it is likely not feasible to continue running through insecticide chemistries as quickly as we historically have. Here, we will explore

some IPM and IRM strategies that could play a vital role in maintaining the efficacy and sustainability of insecticide use in agriculture.

3.1 Areawide Pest Management

Proper use of IPM techniques will likely continue to be vital to sustainable insect management in the future, and will thus play a role in insecticide resistance management. One potential IPM method that could have promise to aid in resistance management, especially in areas where continuous production occurs, is the implementation of areawide management programs (Zalucki et al., 2009). This type of program could help combat the impacts of continuous host crop presence in these areas. For example, if a region only allowed a certain crop species to be grown during a set pre-determined portion of the year, this could help to impose an offseason for insect pests that are specific to that crop, reducing the danger of continuous selection imposed on that pest. An example of this can be found for *Brassicac*s grown in southern Queensland in Australia. A three-month production break in this area seemed to help disrupt the pest lifecycle and reduce pest pressure, and this break was associated with reduced LD50s for some pesticides in diamondback moth (*Plutella xylostella*) (Hargreaves, 1996). This type of program has promise to aid in management of otherwise difficult to manage pests that are relatively host-specific, though its success would require widespread adoption of the program. As a result, it may be necessary for this to be achieved through policy choices that ensure compliance with these regulations. The use of areawide pest management tactics has also shown promise to help manage codling moth in a variety of areas, including British Columbia, Washington, Oregon, and California (Knight, 2008; Thistlewood & Judd, 2019). Research has also shown that modeling based on the

occurrence and location of surrounding potential habitat for insect pests can be used to effectively model the danger of insect damage in a crop (Tabuchi et al., 2017). By mapping land use within a 300-m radius surrounding several rice paddies and identifying sources of sorghum plant bugs (*Stenotus rubrovittatus*), the researchers were able to produce a model that was consistent with observed damage to the paddies. This type of modeling would be important to designing effective areawide management plans and allowing producers to make informed decisions for planting and pest management.

Another common tactic utilized in areawide pest management programs is the sterile insect technique, as discussed by Thistlewood and Judd (2019) for codling moth control. The sterile insect technique is a process by which large numbers of sterile males are reared and released systematically over a sustained time period in order to manipulate pest reproduction (Vreysen et al., 2006). This tactic has proven useful in controlling and even eradicating certain pests, such as the eradication of the new world screwworm, *Cochliomyia hominivorax*, from North and Central America during the second half of the 20th century (Scott et al., 2017). More recently, the sterile insect technique was also utilized to successfully eradicate the pink bollworm, *Pectinophora gossypiella*, from the United States and northern Mexico (Tabashnik et al., 2020). While this technique has promise, it requires extensive economic input to mass rear and release sterilized individuals that are reproductively competitive with the native population. The success of this method also depends on the reproductive biology of the target insect. In order for this technique to be useful, females of the target species should only mate once (Benedict, 2021). This ensures that when a female mates with a sterile male she will produce no

offspring. This compares to a species where females mate multiple times, and mating with a sterile male would not prevent the female from mating again with a fertile male.

3.2 Managing Host Stress

Another potentially important step forward in the future of IPM is moving away from the concept of insect control and toward the concept of host stress management (Peterson et al., 2018). By taking this approach to IPM, it may be possible to reduce the selection pressure placed on insects by simply reducing the need for control tactics that would impose it. An integral part of this approach is improving the tolerance of plants to insect damage. In the quest to eliminate the danger of plant damage by insects, we have focused heavily on the biology of the pests, rather than that of the plants. This has led to plant tolerance being largely unstudied (Peterson et al., 2017). The primary benefit of host plant tolerance as an IPM strategy is that it is not likely to impose selection pressure on the insect (Fornoni, 2011).

Plant tolerance is a characteristic that allows the plant to compensate for the damage caused by insect herbivory without negatively impacting (i.e., selection pressure) the insect directly. Additionally, improved plant tolerance means that insect populations do not need to be maintained at the same low levels as in susceptible plants, thereby reducing the need for the use of insect control tactics. Though host tolerance seems to have promise in management of crop insect pests, it has not been well studied, and the mechanisms by which tolerance occurs are not well understood (Fornoni, 2011; Peterson et al., 2017). One mechanism of plant tolerance to insect damage that has been observed is increased plant growth in response to herbivore feeding that allows the plant to overcome the damage. This has been seen in raspberry, where aphid feeding promoted

increased plant growth rates (Johnson et al., 2012) and in sugarcane, where increased growth of tops, roots, and stubble resulted from root feeding by white grubs (Allsopp & Cox, 2002). This is an area that would benefit greatly from further research, and it could have great promise for improving insect pest management.

3.3 Insecticide Resistance Mechanisms

An important aspect of moving forward in insecticide resistance management will be further researching the nature and development of resistance. By studying what has led to the development of resistance in the past, we can make more educated decisions about how to manage it into the future. One avenue of research that has seen extensive study and could have promise for aiding insecticide resistance management in the future is researching the molecular basis of resistance in various insects to various insecticides. Insecticide resistance can occur through several processes in the insect: increased metabolic detoxification, target site alteration, and reduced penetration of the insect cuticle (Karunaratne, De Silva, Weeraratne, & Surendran, 2018). A variety of molecular genetics techniques have been used to study these phenomena, including RNA interference (RNAi) and the CRISPR/Cas system. RNAi has been utilized to knock down the expression of certain metabolic enzymes in insects to study the role they play in metabolizing insecticides (Homem & Davies, 2018). CRISPR/Cas9 has been used to artificially induce mutations like those occurring in field populations of certain resistant insects to confirm the nature of the mutation (Homem & Davies, 2018).

By gaining a better understanding of the mechanisms by which resistance develops, it will be possible to improve management strategies to address that resistance. For example, the mechanism by which resistance occurs may impact whether it could

have an associated fitness cost for the insect. These differences in fitness cost would help to determine the likelihood of various resistance mechanisms becoming less common in the population in the absence of selection. In addition, certain resistance mechanisms can contribute to greater dangers of cross-resistance. Increased metabolic detoxification rates that contribute resistance to one insecticide may also provide resistance to another unrelated product. For example, a study of indoxacarb-resistant diamondback moth found that the insects had increased expression of several metabolic genes and were also cross-resistant to several other insecticides (Zhang et al., 2017). These other insecticides have different target sites in the insect, suggesting that the cross-resistance could have resulted from the increased detoxification of the insecticides. More research to identify resistance mechanisms will further allow informed decisions by those involved in insect management and pesticide research concerning how to best manage and prevent resistance, as well as what dangers may exist of cross resistances in resistant insect species.

3.4 RNAi: A New Method of Insect Management

As technology continues to improve, new avenues for development of insect control practices will be opened. One of these new technologies that has gained traction in the last several years as a new form of insecticide is the use of RNAi. RNAi is a naturally occurring gene regulation process that occurs in many plant and animal species by which a double-stranded RNA molecule serves as a template for cell machinery to break down complementary mRNA in the cell, silencing the associated gene (Agrawal et al., 2003; Balaško et al., 2020). This means that if dsRNA molecules can be produced that are homologous to genes vital for insect survival, these genes could be silenced,

killing the insect. The targeted nature of RNAi for use as an insecticide means that it is very target specific, and thus it is associated with a very minimal chance of non-target effects (Tan et al., 2016; Whyard et al., 2009). Additionally, dsRNA molecules are short-lived in the environment, meaning that they pose minimal risk of pollution (Dubelman et al., 2014; Fischer et al., 2017). It will be important to remember the lessons of the past in the use of RNAi, however. Just as insects have evolved resistance to chemical and biological insecticidal products, it is likely they will be able to evolve resistance to insecticidal RNAi. In fact, corn rootworms resistant to *DvSnf7* dsRNA have been reported from a field in Illinois (Khajuria et al., 2018). Just as with any other insecticidal product, proper IRM techniques will need to be developed and utilized.

The use of RNAi as an insecticide is being explored extensively for use against certain Coleopteran insect pests. One of these pests is the western corn rootworm. There are several RNAi sequences that have been explored for use in rootworm control. The first of these, *DvSnf7*, has been successfully integrated into GM corn hybrids, and it has shown promise for controlling rootworm through host-induced gene silencing (Head et al., 2017). Other gene targets have also been explored, including reproductive genes, such as *Dvbol* and *Dvvgr*, which caused significantly reduced fecundity when silenced in lab testing with transformed plants (Niu et al., 2017) and *Sec23*, a gene involved in the formation of cellular structures (Vélez et al., 2020). Rootworm adults exposed to ds*Sec23* displayed significant mortality at 100 and 1000 nanogram doses in lab-based tests with transformed plants.

Another insect that has been the target of research concerning RNAi-mediated control is Colorado potato beetle. Research has identified 27 target genes that could be

used for RNAi control of potato beetle. These are related to a variety of processes in the growth and development of the insect (Ma et al., 2020). Transgenic potatoes that express *dsEcr* constructs, which target a gene associated with molting, caused significant mortality in early instar larvae that fed for 72 hours in lab tests (Hussain et al., 2019). Other research has shown that utilizing RNAi could help to improve the efficacy of other pesticides or to help overcome resistance that has developed in some populations. The transcription factor *CncC* regulates a variety of detoxification genes in the Colorado potato beetle genome, including many that are associated with resistance to imidacloprid (Gaddelapati et al., 2018). This study showed that knockdown of this transcription factor led to decreased expression of the resistance-associated genes, and that knockdown of these other genes increased susceptibility to imidacloprid. These findings suggest that an RNAi strategy that targeted these genes could improve the efficacy of imidacloprid against potato beetle. Another study found that RNAi targeting of prohibitin, a protein associated with mitochondrial function and transcriptional modulation, can enhance the toxicity of *Cry3Aa* against CPB larvae, displaying a three-fold mortality increase (Ochoa-Campuzano et al., 2013).

While RNAi holds promise as an insect management tool, there are several drawbacks that need to be addressed. Current research indicates that the most effective method of delivering dsRNA constructs for RNAi to insects is through ingestion by the insect (Kunte et al., 2020). Oral delivery of dsRNA molecules for RNAi is highly effective against Coleopteran insects (Joga et al., 2016), and it has shown promise for use against Lepidopteran and Hemipteran pests, though the gene suppression was not as highly efficacious as the Coleopteran tests (Li et al., 2015). In addition, the short

environmental persistence of RNA molecules could mean there is danger of premature degradation, particularly for RNAi utilized through broadcast applications (Kunte et al., 2020). All in all, RNAi has promise as a novel insecticide to diversify the options producers have for IPM programs. However, further research will be required to improve the necessary technologies for its production and improve our understanding of its mechanisms and properties. It will also be important to determine the adaptations that could allow insects to develop resistance to its use.

There will not be any one final answer to the problem of insecticide resistance management. Although pest management methods and the technology that supports them are always improving, it will be necessary to utilize diverse and integrated tactics for pest management. Continuing to replace older insect management practices with the new and exciting technology on the market will likely result in insects adapting to that new management tactic just as they have adapted to many of the tactics used against them in the past. We must change our way of thinking if we want to escape the pesticide treadmill and reach a point of sustainable pest management. Greater focus on utilizing integrated management tactics, such as utilizing areawide management programs, will be an important part of this process. Further research in plant tolerance will also help to reduce dependence on insecticides, by helping to reduce the need for insect control tactics. In addition to diversifying management tactics, new technologies like RNAi and CRISPR/Cas will allow us to improve our understanding of resistance and how it can be managed. All these factors will likely play an important role in supporting sustainable insect management into the future.

References

- Agrawal, N., Dasaradhi, P. V. N., Mohammed, A., Malhotra, P., Bhatnagar, R. K., & Mukherjee, S. K. (2003). RNA Interference: Biology, Mechanism, and Applications. *Microbiology and Molecular Biology Reviews*, 67(4), 657–685. <https://doi.org/10.1128/membr.67.4.657-685.2003>
- Allsopp, P. G., & Cox, M. C. (2002). Sugarcane clones vary in their resistance to sugarcane whitegrubs. *Australian Journal of Agricultural Research*, 53(10), 1111–1136. <https://doi.org/10.1071/AR02035>
- Balaško, M. K., Mikac, K. M., Bažok, R., & Lemic, D. (2020, September 1). Modern techniques in colorado potato beetle (*Leptinotarsa decemlineata* say) control and resistance management: History review and future perspectives. *Insects*. MDPI AG. <https://doi.org/10.3390/insects11090581>
- Benedict, M. Q. (2021). Sterile Insect Technique: Lessons From the Past. *Journal of Medical Entomology*. <https://doi.org/10.1093/jme/tjab024>
- Dubelman, S., Fischer, J., Zapata, F., Huizinga, K., Jiang, C., Uffman, J., ... Carson, D. (2014). Environmental fate of double-stranded RNA in agricultural soils. *PLoS ONE*, 9(3), 1–7. <https://doi.org/10.1371/journal.pone.0093155>
- Fischer, J. R., Zapata, F., Dubelman, S., Mueller, G. M., Uffman, J. P., Jiang, C., ... Levine, S. L. (2017). Aquatic fate of a double-stranded RNA in a sediment--water system following an over-water application. *Environmental Toxicology and Chemistry*, 36(3), 727–734. <https://doi.org/10.1002/etc.3585>
- Fornoni, J. (2011). Ecological and evolutionary implications of plant tolerance to herbivory. *Functional Ecology*, 25(2), 399–407. <https://doi.org/10.1111/j.1365-2435.2010.01805.x>
- Gaddelapati, S. C., Kalsi, M., Roy, A., & Palli, S. R. (2018). Cap 'n' collar C regulates genes responsible for imidacloprid resistance in the Colorado potato beetle, *Leptinotarsa decemlineata*. *Insect Biochemistry and Molecular Biology*, 99(April), 54–62. <https://doi.org/10.1016/j.ibmb.2018.05.006>
- Hargreaves, J. (1996). *Insecticide resistance and insecticide management for three vegetable pests in South East Queensland*. Gordon, NSW. <https://ausveg.com.au/app/data/technical-insights/docs/VG021.pdf>
- Head, G. P., Carroll, M. W., Evans, S. P., Rule, D. M., Willse, A. R., Clark, T. L., ... Meinke, L. J. (2017). Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. *Pest Management Science*, 73(9), 1883–1899. <https://doi.org/10.1002/ps.4554>

- Homem, R. A., & Davies, T. G. E. (2018, June 1). An overview of functional genomic tools in deciphering insecticide resistance. *Current Opinion in Insect Science*. Elsevier Inc. <https://doi.org/10.1016/j.cois.2018.04.004>
- Hussain, T., Aksoy, E., Çalışkan, M. E., & Bakhsh, A. (2019). Transgenic potato lines expressing hairpin RNAi construct of molting-associated EcR gene exhibit enhanced resistance against Colorado potato beetle (*Leptinotarsa decemlineata*, Say). *Transgenic Research*, 28(1), 151–164. <https://doi.org/10.1007/s11248-018-0109-7>
- Joga, M. R., Zotti, M. J., Smaghe, G., & Christiaens, O. (2016, November 17). RNAi efficiency, systemic properties, and novel delivery methods for pest insect control: What we know so far. *Frontiers in Physiology*. Frontiers Media S.A. <https://doi.org/10.3389/fphys.2016.00553>
- Johnson, S. N., Young, M. W., & Karley, A. J. (2012). Protected raspberry production alters aphid-plant interactions but not aphid population size. *Agricultural and Forest Entomology*, 14(2), 217–224. <https://doi.org/10.1111/j.1461-9563.2011.00561.x>
- Karunaratne, P., De Silva, P., Weeraratne, T., & Surendran, N. (2018). Insecticide resistance in mosquitoes: Development, mechanisms and monitoring. *Ceylon Journal of Science*, 47(4), 299. <https://doi.org/10.4038/cjs.v47i4.7547>
- Khajuria, C., Ivashuta, S., Wiggins, E., Flagel, L., Moar, W., Pleau, M., ... Clark, T. (2018). Development and characterization of the first dsRNA-resistant insect population from western corn rootworm, *Diabrotica virgifera virgifera* LeConte. *PLOS ONE*, 13(5), e0197059. <https://doi.org/10.1371/journal.pone.0197059>
- Knight, A. L. (2008). Codling moth areawide integrated pest management. In *Areawide Pest Management: Theory and Implementation* (pp. 159–190). <https://doi.org/10.1079/9781845933722.0159>
- Kunte, N., McGraw, E., Bell, S., Held, D., & Avila, L. A. (2020). Prospects, challenges and current status of RNAi through insect feeding. *Pest Management Science*, 76(1), 26–41. <https://doi.org/10.1002/ps.5588>
- Li, H., Guan, R., Guo, H., & Miao, X. (2015). New insights into an RNAi approach for plant defence against piercing-sucking and stem-borer insect pests. *Plant Cell and Environment*, 38(11), 2277–2285. <https://doi.org/10.1111/pce.12546>
- Ma, M., He, W., Xu, S., Xu, L., & Zhang, J. (2020, February 1). RNA interference in Colorado potato beetle (*Leptinotarsa decemlineata*): A potential strategy for pest control. *Journal of Integrative Agriculture*. Chinese Academy of Agricultural Sciences. [https://doi.org/10.1016/S2095-3119\(19\)62702-4](https://doi.org/10.1016/S2095-3119(19)62702-4)
- Niu, X., Kassa, A., Hu, X., Robeson, J., McMahan, M., Richtman, N. M., ... Wu, G. (2017). Control of Western Corn Rootworm (*Diabrotica virgifera virgifera*)

- Reproduction through Plant-Mediated RNA Interference. *Scientific Reports*, 7(1), 1–13. <https://doi.org/10.1038/s41598-017-12638-3>
- Ochoa-Campuzano, C., Martínez-Ramírez, A. C., Contreras, E., Rausell, C., & Real, M. D. (2013). Prohibitin, an essential protein for Colorado potato beetle larval viability, is relevant to *Bacillus thuringiensis* Cry3Aa toxicity. *Pesticide Biochemistry and Physiology*, 107(3), 299–308. <https://doi.org/10.1016/j.pestbp.2013.09.001>
- Peterson, R. K. D., Higley, L. G., & Pedigo, L. P. (2018). Whatever Happened to IPM? *American Entomologist*, 64(3), 146–150. <https://doi.org/10.1093/ae/tmy049>
- Peterson, R. K. D., Varella, A. C., & Higley, L. G. (2017). Tolerance: The forgotten child of plant resistance. *PeerJ*, 2017(10), e3934. <https://doi.org/10.7717/peerj.3934>
- Scott, M. J., Concha, C., Welch, J. B., Phillips, P. L., & Skoda, S. R. (2017). Review of research advances in the screwworm eradication program over the past 25 years. *Entomologia Experimentalis et Applicata*, 164(3), 226–236. <https://doi.org/10.1111/eea.12607>
- Sparks, T. C. (2013, September 1). Insecticide discovery: An evaluation and analysis. *Pesticide Biochemistry and Physiology*. Academic Press. <https://doi.org/10.1016/j.pestbp.2013.05.012>
- Tabashnik, B. E., Liesner, L. R., Ellsworth, P. C., Unnithan, G. C., Fabrick, J. A., Naranjo, S. E., ... Carrière, Y. (2020). Transgenic cotton and sterile insect releases synergize eradication of pink bollworm a century after it invaded the United States. *Proceedings of the National Academy of Sciences of the United States of America*, 118(1). <https://doi.org/10.1073/pnas.2019115118>
- Tabuchi, K., Murakami, T., Okudera, S., Furihata, S., Sakakibara, M., Takahashi, A., & Yasuda, T. (2017). Predicting potential rice damage by insect pests using land use data: A 3-year study for area-wide pest management. *Agriculture, Ecosystems and Environment*, 249, 4–11. <https://doi.org/10.1016/j.agee.2017.08.009>
- Tan, J., Levine, S. L., Bachman, P. M., Jensen, P. D., Mueller, G. M., Uffman, J. P., ... Beevers, M. H. (2016). No impact of DvSnf7 RNA on honey bee (*Apis mellifera* L.) adults and larvae in dietary feeding tests. *Environmental Toxicology and Chemistry*, 35(2), 287–294. <https://doi.org/10.1002/etc.3075>
- Thistlewood, H. M. A., & Judd, G. J. R. (2019, September 10). Twenty-five years of research experience with the sterile insect technique and area-wide management of codling moth, *Cydia pomonella* (L.), in Canada. *Insects*. MDPI AG. <https://doi.org/10.3390/insects10090292>
- Vélez, A. M., Fishilevich, E., Rangasamy, M., Khajuria, C., McCaskill, D. G., Pereira, A. E., ... Siegfried, B. D. (2020). Control of western corn rootworm via RNAi traits in

maize: lethal and sublethal effects of Sec23 dsRNA. *Pest Management Science*, 76(4), 1500–1512. <https://doi.org/10.1002/ps.5666>

- Vreysen, M. J. B., Hendrichs, J., & Enkerlin, W. R. (2006). The sterile insect technique as a component of sustainable area-wide integrated pest management of selected horticultural insect pests. *Journal of Fruit and Ornamental Plant Research*, 14, 107–130.
- Whyard, S., Singh, A. D., & Wong, S. (2009). Ingested double-stranded RNAs can act as species-specific insecticides. *Insect Biochemistry and Molecular Biology*, 39(11), 824–832. <https://doi.org/10.1016/J.IBMB.2009.09.007>
- Zalucki, M. P., Adamson, D., & Furlong, M. J. (2009). The future of IPM: whither or wither? *Australian Journal of Entomology*, 48(2), 85–96. <https://doi.org/10.1111/j.1440-6055.2009.00690.x>
- Zhang, S., Zhang, X., Shen, J., Li, D., Wan, H., You, H., & Li, J. (2017). Cross-resistance and biochemical mechanisms of resistance to indoxacarb in the diamondback moth, *Plutella xylostella*. *Pesticide Biochemistry and Physiology*, 140, 85–89. <https://doi.org/10.1016/j.pestbp.2017.06.011>