Pesticide Mixtures

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1. Introduction

A pesticide mixture is when two or more pesticides (in this case, insecticides and/or miticides) are combined into a single spray solution (Cloyd 2001a). A pesticide mixture entails exposing individuals in an arthropod (insect and/or mite) pest population to each pesticide simultaneously (Tabashnik 1989; Hoy 1998). Pesticide mixtures may be more effective against certain life stages including eggs, larvae, nymphs, and adults of arthropod pests than individual applications (Blümel and Gross 2001) although this may vary depending on the rates used and formulation of the pesticides mixed together (Blümel and Gross 2001).

There is already wide-spread use of pesticide mixtures associated with greenhouse and nursery operations world-wide, partly because combinations of selective pesticides may be required in order to deal with the arthropod pest population complex present in the crop (Tabashnik 1989; Bynum et al. 1997; Helyer 2002; Ahmad 2004; Warnock and Cloyd 2005; Cloyd 2009; Khajehali et al. 2009). Typically, two pesticides are mixed together; however, it has been demonstrated that three or more pesticides may be combined into a spray solution to target different insect and/or mite pests (Cloyd 2009). This book chapter discusses the benefits and concerns associated with pesticide mixtures, how pesticide mixtures may mitigate resistance, and the impact of pesticide mixtures on natural enemies.

2. Benefits associated with pesticide mixtures

Pesticide mixtures may enhance arthropod pest population suppression due to either synergistic interaction or potentiation between or among pesticides that are mixed together (All et al. 1977; Curtis 1985; Comins 1986; Ware and Whitacre 2004; Warnock and Cloyd 2005; Cloyd et al. 2007). Synergism refers to the toxicity of a given pesticide being enhanced by the addition of a less or non-toxic pesticide, or other compound such as a synergist (Chapman and Penman 1980; Ware and Whitacre 2004; Ahmad 2004). Potentiation involves an increased toxic effect on an arthropod pest population when mixing two compounds together, which by themselves are harmful to arthropod pests (Chapman and Penman 1980; Marer 1988; Ahmad 2004; Ahmad 2009).

The primary benefit of mixing pesticides together is a reduction in the number of applications required, which decreases labor costs (Cabello and Canero 1994; Blackshaw et

al. 1995). Furthermore, pesticide mixtures may result in higher mortality of arthropod pest populations than if either pesticide were applied separately (Warnock and Cloyd 2005). Studies have demonstrated that pesticide mixtures increase efficacy against insect pests such as the western flower thrips, *Frankliniella occidentalis* Pergande (Cloyd 2003) and whiteflies (Brownbridge et al. 2000) compared to separate applications of each pesticide. For example, when permethrin (pyrethroid) is mixed with chlorpyrifos or methyl parathion (organophosphates), toxicity increases against certain insect pests (All et al. 1977; Koziol and Witkowski 1982). Pesticide mixtures associated with pyrethroid-based insecticides have been shown to potentiate the activity of the microbial, *Bacillus thuringiensis* Berliner subsp. *galleriae* against the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Salma et al. 1984), and *B. thuringiensis* subsp. *kurstaki* against the fall armyworm, *S. frugiperda* (J. E. Smith) (Habib and Garcia 1981). Pesticide mixtures containing the botanical insecticide, pyrethrum appear to increase the efficacy of *B. thuringiensis* subsp. *kurstaki* against the fall webworm, *Hyphantria cunea* (Drury) (Morris 1972), and a combination of spinosad (spinosyn) and chlorpyrifos provided the best control of four species of *Liposcelis* (psocids) (Nayak and Daglish 2007).

Many studies have evaluated the effects of pesticide mixtures in suppressing populations of agricultural insect pests (All et al. 1977; Koziol and Witkowski 1982; Salma et al. 1984; Moar and Trumble 1987; Nayak and Daglish 2007) whereas there is less information associated with pesticides mixtures, and insect and mite pests of ornamental crops (Warnock and Cloyd 2005). However, Warnock and Cloyd (2005) demonstrated that all two, three, and four-way pesticide mixtures involving abamectin (macrocyclic lactone), bifenazate (carbazate), azadirachtin (limonoid insect growth regulator), and imidacloprid (neonicotinoid) along with spinosad did not affect suppression (based on percent mortality) of western flower thrips populations. This indicated that antagonism was not an issue in any of the pesticide mixtures. Cloyd et al. (2007) found that nearly all the two and three-way combinations associated with the pesticides acetamiprid (neonicotinoid), bifenazate, buprofezin (thiadiazine), and chlorfenapyr (pyrrole) exhibited no antagonistic activity with all the pesticide mixtures efficacious (based on percent mortality) against populations of the sweet potato whitefly B-biotype (*Bemisia tabaci* Gennadius) and the twospotted spider mite, *Tetranychus urticae* Koch. Mixtures of the insecticide/miticide abamectin and the fungicide triforine provided 95% control of twospotted spider mite adults, larvae, and eggs (Wang and Taashiu 1994). Improved control of the twospotted spider mite was obtained with a mixture of the miticides fenpyroximate (pyrazole) and propargite (organosulfur) compared to when both miticides were applied separately (Herron et al. 2003).

3. Concerns associated with pesticide mixtures

Although there are benefits associated with pesticide mixtures, potential problems need to be considered when two or more pesticides are mixed together. These include plant injury (=phytotoxicity), pesticide incompatibility (Cloyd 2001b), and antagonism (Lindquist 2002). Antagonism occurs when mixing two or more pesticides together results in reduced efficacy (based on percent mortality) compared to separate applications of each pesticide or when the combined toxicity of two materials when applied together is less than the sum of the toxicities of the materials when applied separately (Lindquist 2002). Antagonism may compromise the efficacy of insecticides and/or miticides under field conditions (Khajehali et al. 2009). For example, mixing together the miticide bifenazate with the organophosphate

insecticide chlorpyrifos, and carbamate insecticides carbaryl, methomyl, and oxamyl decreased the efficacy of bifenazate against the twospotted spider mite indicating the occurrence of antagonism (Van Leeuwen et al. 2007; Khajehali et al. 2009). However, these effects may vary depending on the insect or mite strain (or strains), physiology, and resistance mechanisms present in the population (Ahmad 2004).

Incompatibility is a physical condition by which pesticides do not mix properly to form a homogenous solution or suspension. Instead, flakes, crystals, or oily clumps form or there is a noticeable separation. Incompatibility may be due to the chemical and/or physical properties of the pesticides, impurities in the water, or the types of pesticide formulations being mixed together (Marer 1988). In order to determine incompatibility (or compatibility) of a pesticide mixture, a 'jar test' should be conducted in which a representative sample of a pesticide mixture solution is collected in a glass jar and then allowed to remain stationary for approximately 15 minutes. If the solution is uniform or homogenous, then the pesticides are compatible; however, if there is clumping or separation, then the pesticides are not compatible with each other (Marer 1988).

4. Pesticide mixtures and resistance mitigation

It has been proposed that pesticide mixtures may delay the onset of resistance developing in arthropod pest populations (Skylakakis 1981; Mani 1985; Mallet 1989; Bielza et al. 2009). The implementation of pesticide resistance mitigating strategies is important for preserving the effectiveness of currently available pesticides (Hoy 1998). However, there is minimal evidence to suggest that pesticide mixtures may actually mitigate the onset of resistance (Immaraju et al. 1990).

Mixing pesticides with different modes of action may delay resistance developing within arthropod pest populations because the mechanism(s) required to resist each pesticide in the mixture may not be wide-spread or exist in arthropod pest populations (Georghiou 1980; Curtis 1985; Mani 1985; Mallet 1989; Ahmad 2004). As such, it may be difficult for individuals in the arthropod pest population to develop resistance to several modes of action simultaneously (Brattsten et al. 1986; Mallet 1989; Stenersen 2004; Yu 2008). Those arthropods present in the population resistant to one or more pesticides would likely succumb to the other pesticide in the mixture as long as pesticides with different modes of action are mixed together (Georghiou 1980; Mallet 1989; Yu 2008). For example, Crowder et al. (1984) reported that a mixture of chlordimeform (formamidine) with permethrin, delayed resistance development in populations of the tobacco budworm, *Heliothis virescens* (F.). However, pesticide mixtures may not always delay resistance (Burden et al. 1960). Attique et al. (2006) indicated that pesticide mixtures were less effective in delaying resistance associated with diamondback moth, *Plutella xylostella* (L.) populations than when applying insecticides separately. Furthermore, this approach may risk selecting for a detoxification mechanism that could allow survival to both pesticides (Stenersen 2004), and may actually enhance overall "selection pressure," thus accelerating the evolution of resistance (Curtis 1985; Via 1986; Brattsten et al. 1986).

The effect of pesticide mixtures is, however, unpredictable because differences in the mode of action do not necessarily insure a lack of common resistance mechanisms and may only reflect the specificity associated with enzymes responsible for detoxification (Sawicki 1981; Yu 2008). Moreover, the effects of pesticide mixtures may differ depending on the arthropod pest population as a result of peculiarities associated with species, strain, and even biotype

(Sawicki 1981; Georghiou and Taylor 1986; Ishaaya 1993). These differences could be related to physiology and the resistance mechanisms present in the population (Georghiou and Taylor 1977a; Brattsen et al. 1986). Also, resistance mechanisms typically don't respond to "selection pressure" or frequency of pesticide applications the same way based on the pesticide being applied. In fact, some resistance mechanisms may negate the advantages of pesticide mixtures (Tabashnik 1989; Stenersen 2004).

One aspect of pesticide mixtures is the opportunity for complex interactions including synergism or antagonism. Two active ingredients may compete for or inhibit the same enzyme (e.g., esterase), which could increase the toxicity of the pesticide mixture (Kulkrani and Hodgson 1980). Synergism may occur when one pesticide interferes with the metabolic detoxification of another pesticide (Corbett 1974; Kulkrani and Hodgson 1980). Certain organophosphate insecticides bind to the active site associated with esterase enzymes responsible for detoxification of pyrethroid-based insecticides (Kulkarni and Hodgson 1980; Ascher et al. 1986; Ishaaya et al. 1987; Bynum et al. 1997; Gunning et al. 1999; Ahmad 2004; Zalom et al. 2005; Ahmad et al. 2008; Ahmad 2009), and so organophosphate insecticides may be considered useful synergists for pyrethroids (Chapman and Penman 1980; Brattsten et al. 1986; Ishaaya et al. 1987; Gunning et al. 1999; Martin et al. 2003; Zalom et al. 2005; Attique et al. 2006). This is one of the main reasons why manufacturing companies formulate organophosphate and pyrethroid-based insecticide mixtures to manage arthropod pest complexes and counteract resistance (Ahmad 2004). Examples of commercially available products for use in greenhouse and/or nursery production systems include Tame/Orthene TR [fenpropathrin (pyrethroid) and acephate (organophosphate); Whitmire Micro-Gen Research Laboratories, Inc., St. Louis, MO] and Duraplex® TR [chlorpyrifos (organophosphate) and cyfluthrin (pyrethroid); Whitmire Micro-Gen Research Laboratories, Inc., St. Louis, MO]. Certain carbamate insecticides have also been reported to synergize the effects of pyrethroid-based insecticides. The carbamate insecticides methiocarb, pirimicarb and oxamyl, and even the fungicide propamocarb have been shown to synergize the efficacy (based on percent mortality) of the pyrethroid-based insecticide acrinathrin against the western flower thrips (Bielza et al. 2007; Bielza et al. 2009).

However, continued use of these types of pesticide mixtures may result in resistance to both modes of activity by arthropod pest populations, especially those that have the capacity of developing multiple resistance, which refers to an arthropod pest population resistant to pesticides with discrete modes of action or across chemical classes affiliated with the expression of different resistance mechanisms (Forgash 1984; Comins 1986; Georghiou 1986; Brattsen et al. 1986; Metcalf 1989; Attique et al. 2006; Ahmad et al. 2008).

As with applications of individual pesticides, it is important to only mix together pesticides with different modes of action or those that affect different biochemical processes in order to mitigate resistance developing in arthropod pest populations (Cranham and Helle 1985; Cloyd 2009). For example, acephate and methiocarb should not be mixed together because despite being in different chemical classes (organophosphate and carbamate) both have identical modes of action. Acephate and methiocarb block the action of acetylcholinesterase, an enzyme that deactivates acetylcholine, which is responsible for activating acetylcholine receptors. This then allows nerve signals to migrate through the central nervous system. Both acephate and methiocarb inhibit the action of acetylcholinesterase by attaching to the enzyme (Ware and Whitacre 2004; Yu 2008). Similarly, although the active ingredients acequinocyl, pyridaben, and fenpyroximate are in different chemical classes; napththoquinone, pyridazinone, and phenoxypyrazole, respectively all three are classified as mitochondrial electron transport inhibitors (METI). These active ingredients either inhibit

nicotinamide adenine dinucleotide hydride (NADH) dehydrogenase (complex I) associated with electron transport, acting on the NADH CoQ reductase, or bind to the quinone oxidizing (Q_o) center or cytochrome bc_1 (complex III) of the mitochondria respiratory pathway. This reduces energy production by preventing the formation of adenosine triphosphate or ATP (Hollingworth and Ahammadsahib 1995; Yu 2008).

Pesticide mixtures may mitigate the onset of resistance under the following assumptions: 1) resistance associated with each pesticide in a mixture is monogenic (resistance resulting from the expression of a single gene) and independently genetically controlled (Curtis 1985; Tabashnik 1989). In addition, there is no cross resistance among individuals in the arthropod pest population to the pesticides used in the mixture (Mani 1985; Comins 1986; Tabashnik 1989; Tabashnik 1990). Cross resistance refers to a condition by which resistance to one pesticide confers resistance to another pesticide, even though the arthropod pest population was never exposed to the second pesticide; and insensitivity to pesticides with similar modes of action or in the same chemical class due to a common resistance mechanism or detoxification pathway associated with different pesticides (Cranham and Helle 1985; Georghiou and Taylor 1986; Roush 1993; Pedigo 2002). These conditions occur when there are different target sites and detoxification enzymes affiliated with resistance to the two pesticides. It is possible that under these given circumstances, individuals simultaneously possessing resistance mechanisms to both pesticides will be extremely rare (Curtis 1985; Brattsten et al. 1986; Mallet 1989; Roush 1993); 2) individuals in the arthropod pest population possess resistance genes (alleles) that are exclusively recessive and/or individuals that are doubly-resistant are very rare. Evolution of resistance will be instantaneous if any survivors possess doubly-resistant genes or multiple resistance mechanisms (Curtis 1985; Comins 1986; Tabashnik 1989; Mallet 1989); 3) some individuals in the arthropod pest population are not treated or exposed to the pesticide mixture primarily due to the presence of refugia (Georghiou and Taylor 1977b; Brattsten et al. 1986; Tabashnik 1989; Tabashnik 1990), or there is immigration of and mating with susceptible individuals, which reduces the frequency or proportion of resistant individuals (or resistant genes) in the arthropod pest population (Comins 1977; Georghiou and Taylor 1977b; Tabashnik and Croft 1982; Comins 1986; Georghiou and Taylor 1986; Mallet 1989; Jensen 2000; Stenersen 2004); 4) the pesticides mixed together have equal persistence so that any individuals in the arthropod pest population are not exposed to just one pesticide for an extended length of time (Forgash 1984; Curtis 1985; Tabashnik 1989; Tabashnik 1990; Roush 1993); and 5) resistance mechanisms to each pesticide are present at such low frequencies that they may not occur together in any individuals in an arthropod pest population (Yu 2008).

The assumptions presented above, in nearly all instances, are not realistic. For example, pesticide mixtures may, in fact, promote the expression of multiple resistance, which could extend across other chemical classes resulting in specific arthropod pest populations being very difficult to manage (Forgash 1984; Brattsten et al. 1986; Ahmad 2004; Attique et al. 2006). Furthermore, multiple evolutionary pathways may exist that eventually result in a pesticide-resistant arthropod pest population (Metcalf 1980; Georghiou 1983; Brattsten et al. 1986; Ishaaya 1993). Although pesticide mixtures may delay resistance due to target site insensitivity, which is usually specific to a certain class of pesticides, the use of pesticide mixtures enhances the selection for increased expression of metabolic enzymes that can simultaneously detoxify both pesticides (Roush and McKenzie 1987; Roush and Daly 1990; Roush and Tabashnik 1990; Stenersen 2004). Also, cross and multiple resistance may occur among some pesticides with similar modes of action (Stenersen 2004). Therefore, selecting

for high levels of detoxification enzyme expression jeopardizes the usefulness of all pesticides, even those with new modes of action to which the arthropod pest population has never been previously exposed (Tabashnik 1989; Soderlund and Bloomquist 1990).

Additional problems associated with the assumptions of using pesticide mixtures to mitigate resistance are that the frequency of doubly-resistant individuals or those with multiple resistance mechanisms in the arthropod pest population may be extensive (Tabashnik 1989). This may be due to a history of pesticide exposure associated with selection for resistance in previous arthropod pest generations, which could imply that there may be some background levels of resistant traits or mechanisms in the arthropod pest population for each pesticide used in the mixture (Georghiou and Taylor 1977a). Also, there is usually no refuge to preserve susceptible individuals (Georghiou and Taylor 1986; Tabashnik 1989), particularly in enclosed ornamental production systems.

Is the use of pesticide mixtures the most appropriate way to extend their usefulness, or is it preferable to apply them individually? Pesticide mixtures, in fact, may be expensive, especially if the pesticides that are mixed together are used at the highest recommended label rate (Curtis 1985; Comins 1986; Mallet 1989; Attique et al. 2006). As such, a common practice is too use reduced rates of each pesticide in the mixture although this may not actually mitigate resistance developing in arthropod pest populations (Suthert and Comins 1979). More sophisticated uses of pesticide mixtures will require a thorough understanding of their interactions in order to optimize the dosage at below label rates when the components (active and inert ingredients) act synergistically (Tabashnik 1989; Attique et al. 2006). Pesticide mixtures may be an effective means of mitigating resistance as long as there is a high level of dominance in the arthropod pest population and immigration of susceptible individuals is prevalent (Mani 1985; Georghiou and Taylor 1986). Based on population genetic models, pesticide mixtures may effectively suppress resistance genes that are recessive and accord resistance to only one pesticide. However, it is possible that pesticide mixtures will select for dominant genes, which confer cross resistance (Tabashnik 1989).

The rate of resistance development in an arthropod pest population to two or more pesticides in a mixture may take longer than when the pesticides are applied separately (National Research Council 1986) although resistance to a pesticide mixture may occur at a similar rate as when the pesticides are applied individually (Kable and Jeffery 1980). The advantages of a pesticide mixture will only be sustained as long as resistance is not fully-dominant (Curtis 1985). Because the reliability of the pesticide mixture strategy depends on several assumptions, applying pesticides individually, or rotating those with different modes of action or that act on different target sites may be a more appropriate strategy (Roush 1993).

5. Pesticide mixtures and natural enemies

The use of pesticide mixtures with a broad-spectrum of arthropod pest activity and multiple modes of action may negatively impact biological control agents or natural enemies more so than separate applications of pesticides (Ahmad et al. 2004). However, only a few studies have evaluated the direct and indirect effects of pesticide mixtures on natural enemies and these primarily involve predatory mites. Lash et al. (2007) found that *Neoseiulus cucumeris* (Oudemans) deutonymphs were more sensitive to certain pesticide mixtures involving the insecticide spinosad, the insecticide/miticide abamectin, and the fungicides fenhexamid and

thiophanate-methyl than adults. Predatory mite mortality, in general, associated with the pesticide mixtures was not significantly different from mortality when the pesticides were applied separately (Lash et al. 2007).

Field studies conducted with the predatory mite, *Typhlodromus pyri* Scheuten found that mixtures of the fungicides mancozeb or thiophanate-methyl with the insecticide chlorpyrifos were more harmful to the predatory mite than if the pesticides were applied by themselves (Cross and Berrie 1996). Sterk et al. (1994) determined that the fungicides maneb and mancozeb were moderately toxic to *T. pyri* when applied separately but their effects were diminished when both fungicides were mixed together. Blumel and Gross (2001) indicated no significant differences in the mortality rate or fecundity associated with *Phytoseiulus persimilis* Athias-Henriot females following exposure to the miticide (acaricide) hexythiazox (carboxamide), the fungicide triadimefon, and the insecticide heptenophos (organophosphate) when applied either individually or in mixtures.

Boomathi et al. (2005) evaluated the effects of pesticide mixtures on the parasitoid, *Trichogramma chilonis* Ishii and found that combinations of spinosad with *Bacillus thuringiensis* var. *galleriae* were toxic to adults (based on percent mortality) and inhibited adult emergence. Based on the studies presented above, pesticide mixtures may differentially directly (e.g., immediate mortality) or indirectly (e.g., delay female oviposition) impact natural enemies.

6. Summary

Pesticide mixtures involve combinations of two or more pesticides into a single spray solution. Pesticide mixtures are widely used to deal with the array of arthropod pests encountered in greenhouse and nursery production systems due to the savings in labor costs. Furthermore, the use of pesticide mixtures may result in synergism or potentiation (enhanced efficacy) and the mitigation of resistance (Ahmad 2009). However, antagonism (reduction in efficacy) may also occur due to mixing two (or more) pesticides together. Judicious use of pesticide mixtures or those that may be integrated with biological control agents is especially important because parasitoids and predators (and even microbials such as beneficial bacteria and fungi) can suppress arthropod pest populations irrespective of the arthropod pests' resistance traits or mechanisms (Tabashnik 1986). The use of pesticide mixtures to mitigate resistance must not divert attention from the implementation of alternative pest management strategies including cultural, sanitation, and biological control that can reduce reliance on pesticide mixtures and mitigate pesticide resistance (Georghiou 1983; Metcalf 1983; Tabashnik 1989; Roush 1989; Roush and Tabashnik 1990; Hoy 1998; Denholm and Jespersen 1998). Pesticide mixtures will continue to be an integral component of pest management programs due to the continual need to deal with a multitude of arthropod pests associated with ornamental cropping systems.

7. References

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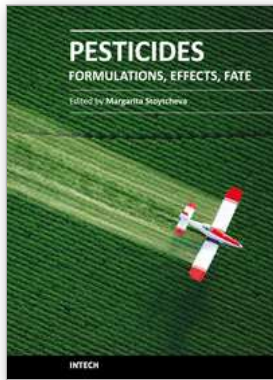
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This book provides an overview on a large variety of pesticide-related topics, organized in three sections. The first part is dedicated to the "safer" pesticides derived from natural materials, the design and the optimization of pesticides formulations, and the techniques for pesticides application. The second part is intended to demonstrate the agricultural products, environmental and biota pesticides contamination and the impacts of the pesticides presence on the ecosystems. The third part presents current investigations of the naturally occurring pesticides degradation phenomena, the environmental effects of the break down products, and different approaches to pesticides residues treatment. Written by leading experts in their respective areas, the book is highly recommended to the professionals, interested in pesticides issues.

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