Effects of Coragen®, a ryanoid insecticide, applied topically to two crop-representative spiders, *Enoplognatha ovata* (Araneae: Theridiidae) and *Tibellus* spp. (Araneae: Philodromidae)

by

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ABSTRACT

Effects of Coragen®, a ryanoid insecticide, applied topically to two crop-representative spiders, *Enoplognatha ovata* (Araneae: Theridiidae) and *Tibellus* spp. (Araneae: Philodromidae)

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The role of spiders as natural enemies can be compromised by their susceptibility to broad-spectrum insecticides. To date the toxic effects of Coragen® (a.i. Chlorantraniliprole) (DuPont Canada), a recently introduced ryanoid insecticide, have not been investigated in spiders. In this thesis, the direct lethal and sublethal effects of Coragen® applied topically to *Enoplognatha ovata*, and *Tibellus* spp., were determined. Coragen® applied at concentrations of 1.125 mg mL$^{-1}$ (corresponding to the manufacturer recommended tank concentrations of 0.5 to 1.0 mg mL$^{-1}$) caused mortality in >50% of the spiders tested. In arena tests sublethal concentrations did not affect the rate at which spiders killed *Drosophila*. The toxicity of Coragen® was enhanced by co-application of piperonyl butoxide (PBO). These results suggest that differences between the ryanodine receptors of target pests and spiders are insufficient to result in selective toxicity and metabolic detoxification of Coragen® by spiders depends on oxidative and/or hydrolytic enzymes.
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No matter how swift the current is, keep paddling and do not let the waterfall consume you.

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Chapter 1

GENERAL INTRODUCTION

1.1 Introduction

It is important to understand the effects of pesticides on spiders. Spiders (Order: Araneae) are an abundant and diverse group of natural enemies (Turnbull, 1973; Wise, 1993; World Spider Catalog, 2015) that have demonstrated the ability to suppress and influence insect populations in a variety of terrestrial ecosystems (Kajak et al., 1968; Mansour et al., 1980; Mansour et al., 1983; Riechert & Bishop, 1990; Carter & Rypstra, 1995). In agroecosystems, these generalist predators prey directly on insect pests and contribute to pest management (Carter & Rypstra, 1995; Greenstone, 1999; Hoefler et al., 2006). This can positively affect crop health and productivity by limiting the number of herbivorous pests (Sunderland, 1999; Hlivko & Rypstra, 2003). However, many pesticides have been found to interfere with the ability of spiders to suppress pests (Pekár, 2012, 2013). Pesticides that interfere with predator-prey relationships within agroecosystems are disruptive to population balances and counterproductive in pest management strategies (Symondson et al., 2002; Pekár, 2012). Consequently, non-selective pesticides can result in greater losses and damage to crops due to pest resurgences and secondary outbreaks if populations of natural enemies are reduced (Ruberson et al., 1998; Yu et al., 2008). Therefore, to conserve, enhance, and support spiders as potential biological control agents (BCAs) in agroecosystems, it is paramount that the effects of pesticides, especially those newly introduced to the market, be investigated on spiders. In this thesis, I characterized the effects of a newly introduced ryanoid insecticide, Coragen (active ingredient = chlorantraniliprole) (CAS # = 500008-45-7), on two crop

### 1.2 Integrated Pest Management

Integrated pest management (IPM) is a crop production and protection technique that depends on a combination of management strategies (Food and Agriculture Organization of the United Nations, 2015). IPM focuses on long-term prevention of pests and their damage through biological, cultural, mechanical/physical, and chemical control measures. Biological control involves the exploitation and support of natural enemies and the employment of BCAs, cultural control involves habitat manipulation (*e.g.* various irrigation and crop rotation practices) and the implementation of hybrid and resistant plant varieties, mechanical/physical control involves the use of traps and barriers (*e.g.* fences and screens), and chemical control involves the employment of pesticides only when absolutely necessary (Higley & Pedigo, 1993; Elliott, 1995; Oerke, 2006). Chemical control in IPM depends on the sensible application of selective pesticides so that environmental quality is maintained and beneficial arthropods, including natural enemies, can continue to perform their ecosystem services effectively (*i.e.* predation and pollination) (Higley & Pedigo, 1993). Successful agricultural practice has long depended on the application of biological, cultural, mechanical/physical, and chemical measures to mitigate losses from pests. Despite the success of many of these countermeasures, insect pests continue to cause millions of dollars in crop damage each year (Oerke, 2006). This is a major concern because demands on food production and efficient agricultural practice are increasing rapidly as the global population continues to grow (Pond et al., 2009).
IPM is crucial for maximizing crop productivity and ensuring that pest populations remain below levels of economic injury (Higley & Pedigo, 1993). The economic injury level (EIL) concept is at the foundation of IPM practice. Instead of focusing on the eradication of pests from crops, it embraces the concept that non-economic damage to crops is tolerable and will not jeopardize yields (Higley & Pedigo, 1993). In IPM, EILs allow for more informed decision-making when determining the most appropriate management tactics (Higley & Pedigo, 1993). For instance, by limiting excessive or inappropriate pesticide use that could be detrimental to the environment and naturally occurring beneficial fauna (i.e. natural enemies and pollinators) (Symondson et al., 2002). IPM aims to minimize disruption to agroecosystems and risk to human health, beneficial and non-target organisms, and the environment by creating unfavourable conditions for pests through biological, cultural, mechanical/physical, and chemical control tactics (Pedigo & Rice, 2014; Akbari et al., 2015; Food and Agriculture Organization of the United Nations, 2015).

1.3 Natural Enemies

Natural enemies are an important component of IPM as they are essential to biological control. Biological control refers to the exploitation or use of an organism to reduce the population density of another organism (van Lanteren, 2012). There are three major types of biological control: conservation (natural) biological control—the protection and support of naturally occurring predators and parasitoids (Waage & Greathead, 1988); importation/classical biological control—the capture and release of natural enemies to control pests in another location; and augmentative biological control—the release of mass-reared natural enemies either in large numbers for immediate reduction in pest damage (inundative) or in small numbers periodically to provide more long-term control (inoculative) (van Lanteren, 2012). Natural enemies are typically predators and
parasitoids. Predatory arthropods currently used globally as BCAs include: predatory mites (Mesostigmata: Phytoseiidae, Parasitidae, Laelapidae), beetles (Coleoptera: Coccinellidae, Staphylinidae, Cybocephalidae), thrips (Thysanoptera: Aeolothripidae, Phlaeothripidae, Thripidae), lacewings (Neuroptera: Hemerobiidae, Chrysopidae), true bugs (Hemiptera: Anthocoridae, Pentatomidae, Geocoridae, Miridae, Nabidae), and predatory flies (Diptera: Muscidae, Cecidomyiidae, Syrphidae) (van Lenteren, 2012). These BCAs directly prey on both the adults and/or immature stages of insect pests (Ruberson et al., 1998; van Lenteren, 2012).

Parasitoids reduce insect pest populations by parasitizing pests (host), usually debilitating or killing them during development. Parasitic wasps and flies (many hymenopteran families, including: Braconidae, Aphelinidae, Encyrtidae, and, Trichogrammatidae; and several dipteran families including: Syrphidae) are some of the most widely used BCAs in augmentative biological control (van Lenteren, 2012). In 2010, they comprised more than 50% of the 230 species of commercially available BCAs in the world (Cock et al., 2010; van Lenteren, 2012). Encarsia (Hymenoptera: Aphelinidae) is one of the most commonly implemented parasitoid genera to control white flies, Trialeurodes spp. (Hemiptera: Aleyrodidae), in greenhouse crop systems (Hoddle et al., 1998; van Lenteren, 2000). However, like many parasitoids, Encarsia are adversely affected by pesticides. Studies by Sohrabi et al. (2012) found that exposure of Encarsia to the neonicotinoid, imidacloprid, caused reduced adult longevity, number of progeny, and adult emergence. Similarly, the pyrethroid, deltamethrin, was also found to negatively affect Encarsia, resulting in 100% mortality at the recommended application rate (1.25 g a.i. 100 L⁻¹) (Delorme et al., 1985). Recent studies by Royauté et al. (2015) found that the jumping spider, Eris militaris (Araneae: Salticidae), displayed personality changes when exposed to sublethal concentrations of the organophosphate, phosmet. Sublethal concentrations of phosmet negatively impacted the way in which spiders explored their
environment and captured prey. Therefore, it is important to understand and endeavour to mitigate the effects of agricultural practices on natural enemies, especially in regard to the application of agricultural insecticides since this can have a detrimental impact on them and their ability to suppress crop pests.

1.4 Importance of spiders as natural enemies and their role in agroecosystems

With over 45,000 species worldwide, spiders are an abundant and diverse group of natural enemies present in nearly every terrestrial ecosystem (Turnbull, 1973; Wise, 1993; World Spider Catalog, 2015). Most frequently found in richly vegetated areas, spiders have also established themselves in more hostile environments such as Arctic islands, desert, tidal zones, and at high altitudes in mountainous regions (Lamoral; 1968; Turnbull, 1973; Gertsch & Riechert, 1976). In view of the breadth of ecological niches and environments spiders occupy, their substantial populations in many agroecosystems are not surprising. Studies have shown that spiders can make up a considerable portion of the natural enemy population in crops, as much as 73% and 81% in cotton in Texas (Sterling et al., 1992) and China (Zhang, 1992), respectively. A survey by Young and Edwards (1990) reported that species numbers range from tens to hundreds in U.S. agroecosystems, with cotton (≤308), soybean (≤262), and alfalfa (≤233) being the most speciose. Spiders also occur in a number of other crop systems, including apple and citrus orchards, rice, taro, spring barley, sweet basil, alfalfa, guar, grain sorghum, corn, peanuts, sugarcane, and vegetables (Itô et al., 1962; Nakasuji et al., 1973; Mansour et al., 1980; Chiverton, 1986; Mansour & Whitcomb, 1986; Oraze & Grigarick, 1989; Riechert & Bishop, 1990; Young & Edwards, 1990; Hoeffler et al., 2006). Owing to their widespread distribution and adaptability to various environments, spiders are arguably one of the most common assemblages of arthropods found in agroecosystems (Riechert &
Lockley, 1984; Young & Edwards, 1990; Greenstone, 1999; Nyffeler & Sunderland, 2003). Their prevalence highlights them as important organisms for further study in the context of biological control in IPM.

Most spider species are generalist (polyphagous) predators, meaning they feed on a wide variety of prey, many of which are responsible for damage to crops (Wise, 1993). Studies by Nyffeler et al. (1994) determined that in U.S. field crops, spiders prey on insects from many insect orders including: Diptera, Hemiptera/Homoptera, Hymenoptera, Coleoptera, Lepidoptera, Orthoptera, Dermaptera, and Neuroptera. This study further concluded that the bulk diet for agroecosystem spiders in the families Tetragnathidae, Oxyopidae, and Salticidae consisted of approximately 70% hemipterans/homopterans and dipterans (Nyffeler et al., 1994). Examples of some common crop pests that spiders preyed upon include: tarnished plant bugs, *Lygus lineolaris* (Palisot) (Hemiptera: Miridae); imported fire ants, *Solenopsis invicta* (Buren) (Hymenoptera: Formicidae); bollworms, *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae); spotted cucumber beetles, *Diabrotica undecimpunctata howardi* (Barber) (Coleoptera: Chrysomelidae); three-cornered alfalfa hoppers, *Spissistilus festinus* (Say) (Hemiptera: Membracidae); boll weevils, *Anthonomus grandis* (Boh) (Coleoptera: Curculionidae); chinch bugs, *Blissus sp.* (Hemiptera: Blissidae); leafhoppers, *Chlorotettix sp.* (Hemiptera: Cicadellidae); fall armyworms, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae); Japanese beetles, *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae); grasshoppers (Order: Orthoptera); and aphids (Hemiptera: Aphididae) (see review in Young & Edwards, 1990). Polyphagy can help maintain predator populations when pest densities are low (Den Boer, 1982; Murdoch et al., 1985; Holt & Lawton, 1994). For spiders and other generalist predators, this often involves subsistence on nonpest prey, including intraspecifics (cannibalism) (Young & Edwards, 1990; Settle et al., 1996; Nilsson, 2001; Wise, 2006). Generally,
organisms are less inclined to emigrate from areas that provide adequate resources (Riechert & Bishop, 1990; Symondson et al., 2002); therefore, generalist predators are less likely to become locally extinct compared to specialist predators once pest populations dwindle (Chang & Kareiva, 1999). Generalists maintain consistent population numbers in agroecosystems and, thus, will always be present to prey on the most locally abundant pests (Den Boer, 1982; Harris, 1990; Chang & Kareiva, 1999; Symondson et al., 2002). Spiders are important generalist predators to consider in IPM because they prey on a wide range of pests and can maintain population stability essential for mitigating pest re-invasions and resurgences.

Over the course of evolution, spiders have developed and adopted a variety of hunting strategies better enabling them to capture and subdue prey. Studies by Post and Riechert (1977) identified as many as 11 guilds based on mode of prey capture, web type, and temporal activity. However, spiders can more easily be identified as either web-builders or active (wandering) hunters (Uetz, 1977; Uetz et al., 1999). Studies have found that direct predation, wasteful killing (i.e., when prey densities are high), secondary cues (e.g. webs, drag lines, feces, prey carcasses), and sheer physical presence are all mechanisms by which spiders prevent or deter pests from herbivory (Carter & Rypstra, 1995; Riechert, 1999; Sunderland, 1999). These mechanisms may vary between guilds, but all work in conjunction with one another to contribute to reduced crop losses. Spiders predominantly target prey of equal or lesser size to themselves (Nyffeler & Benz, 1981; Nentwig & Wissel, 1986), however, some web spinning spiders (Araneae: Araneidae), jumping spiders (Araneae: Salticidae), and crab spiders (Araneae: Thomisidae) have been known to capture prey up to three times their own size (Enders, 1975; Robinson & Valerio, 1977). Spiders have also been found to prey on insect pupae (Lincoln et al., 1967; Buschman et al., 1977; McDaniel & Sterling, 1982). The families Salticidae, Thomisidae, Miturgidae, Oxyopidae, Araneidae, Linyphiidae, and
Anyphaenidae tested positive for *Heliothis virescens* (Lepidoptera: Noctuidae) eggs that had been radioactively labeled with phosphorus (McDaniel & Sterling, 1982). As generalist (polyphagous) predators, spiders possess a non-discriminate feeding strategy that could prove to be an asset and invaluable quality for pest control in IPM.

The ability of spiders to suppress and influence pest populations has been well studied (Kajak *et al.*, 1968; Mansour *et al.*, 1983; Carter & Rypstra, 1995), but pest suppression is not the only role spiders play in ecosystems. Spiders can also serve as a major food source for higher trophic level predators such as insects, birds, fish, lizards, amphibians, and small mammals (Bristowe, 1941; Whitcomb, 1974; Schoener & Toft, 1983; Pacala & Roughgarden, 1984; Schoener & Spiller, 1987; Spiller & Schoener, 1998; Shaw *et al.*, 2002). Additionally, they may also provide a source of food for other organisms in the form of subdued or partially consumed prey (Thornhill, 1975; Nyffeler & Benz, 1980; Heuts & Brunt, 2001). As both predators and prey, spiders contribute significantly to nutrient and energy flow within food webs (van Hook, 1971; Turnbull, 1973; Schoener, 1989; Wise, 1993).

1.4.1 Evidence for pest suppression by spiders and their potential in IPM

In field tests, single spider species (*Trochosa pratensis* (Emerton) (Araneae: Lycosidae); *Florinda coccinea* (Hentz) (Araneae: Linyphiidae); *Pardosa milvina* (Hentz) (Araneae: Lycosidae); *Argiope trifasciata* (Forskal) (Araneae: Araneidae); and *Hogna rabida* (Walckenaer) (Araneae: Lycosidae)) were found to reduce pest numbers and biomass, but not as much as entire spider assemblages (Provencher & Riechert, 1994; Richert & Lawrence, 1997). A diverse assemblage of spiders is believed to ensure more thorough and effective coverage in pest suppression by guaranteeing predators of appropriate size classes, foraging modes, and various phenologies to
complement those of pests (Riechert & Lockley, 1984; Riechert & Bishop, 1990; Riechert, 1999). As a community, the various spider guilds work cohesively to suppress pests and act in limiting the amount of “enemy-free space” (Jeffries & Lawton, 1984). Differences in foliar preference/location, temporal activity, and hunting strategy, explain the wide variation in niches occupied by spiders. Spiders in these niches may be involved in unforeseen and complex interactions. For example, pests may jettison from plants because of cues left behind by web-building spiders, resulting in a source of prey for ground-dwelling/active hunting spider guilds (Sunderland, 1999). Alternatively, a ground-dwelling spider may push prey into the niche of a foliar-dwelling one. Unfortunately, agroecosystems are frequently disturbed environments where the activity of spiders can be easily disrupted. Mowing, plowing, planting, cultivating/harvesting, and pesticide applications are all regular crop occurrences that disturb and reduce spider activity, abundance, and richness (Nyffeler, 1982; Riechert & Lockley, 1984; Carter & Rypstra, 1995; Symondson et al., 2002). This may put strain on the spider community and reduce its effective pest suppression capability since unaffected guilds may be unable to compensate for those being hindered. However, with basic toxicological testing on spiders being relatively unexplored and a lack of understanding for the complex ecological interactions of spiders in field crops, this remains to be resolved (Young & Edwards, 1990; Nyffeler et al., 1994; Pekár, 2012).

Nonlethal mechanisms are also important considerations because they can result in pest suppression even in the absence of spiders. Studies by Hlivko & Rypstra (2003) investigated the nonlethal effects of three different species of wolf spiders (Araneae: Lycosidae), *Pardosa milvina* (Hentz); *Rabidosa rabida* (Walckenaer); *Hogna helluo* (Walckenaer), on damage to soybean leaves by Japanese beetles, *P. japonica*. They found these beetles feed less in an environment that formerly included wolf spiders, indicating that the cues they had left behind (e.g., silk, draglines,
and feces) were still effective deterrents of herbivory. Web-spinning spiders frequently capture prey in their webs that is not consumed because it is either of inappropriate size or simply undesired (Nentwig & Wissel, 1986; Nentwig, 1987; Alderweireldt, 1994). If entangled prey is unable to free itself, dehydration and starvation may ensue leading to death and thus pest suppression without direct predation (Alderweireldt, 1994). Spiders can effectively deter herbivory and crop damage through both lethal and nonlethal mechanisms. Investigating the effects of insecticides on spiders lends insight into how these pest suppression mechanisms might be disrupted and what the resulting consequences may be for spider communities in agroecosystems.

Field studies have shown that spiders are capable of suppressing pests (Mansour et al., 1980; Mansour et al., 1983; Chiverton, 1986; Riechert & Bishop, 1990; Carter & Rypstra, 1995). Studies by Carter & Rypstra (1995) found that leaf damage in controlled soybean crops was reduced in plots where spiders of the species, Achaearanea tepidariorum (Araneae: Theridiidae), were added (augmentation), whereas damage increased in plots where spiders had been removed. Studies conducted by Mansour et al. (1980) found that Egyptian cotton worm populations, Spodoptera littoralis (Boisd.)(Lepidoptera: Noctuidae), could be controlled by pre-existing spider populations (natural biological control) in apple orchards in Israel, as long as spiders remained undisturbed by pesticide applications and harvesting. These studies and others like them (Chiverton, 1986; Riechert & Bishop, 1990;Symondson et al., 2002; Hoefler et al., 2006) suggest that spiders significantly impact pest numbers and play an important role in influencing their population dynamics. By conserving spiders, growers may be able to ensure that agricultural practice is more sustainable. For example, conservation to support biological control by spiders in rice crops in the Hunan region of China saw a 50–60% decline in pesticide use following implementation of in-crop straw and bamboo refugia (USDA, 1982). Refugia provided shelter to the spiders during periods of disturbance.
(i.e., during midday heat, irrigation, and harvesting). Studies by Halley et al. (1996) found that habitat heterogeneity (small patches of grassland) greatly increased the population size of spiders in cereal fields in the U.K. Similar studies by Jmhasly and Nentwig (1995) found spider densities were greatest around sown weed strips in winter wheat in Switzerland. By supporting spider populations and increasing their densities, the potential for pest suppression also increases, reducing the need for pesticide use. These findings highlight the importance of spiders as natural enemies and their contribution to biological control.

Conventional BCAs, especially the parasitoid wasps, have a restricted host range and typically specialize on a single pest species (Bigler et al., 2006). This ensures effective and focused control. However, should a crop become infested with multiple types of pests, then multiple species of parasitoid would need to be implemented to control the outbreak, in turn, becoming more costly (Symondson et al., 2002). Spiders, in contrast, are polyphagous predators that target a variety of pests (Nentwig, 1982; Nyffeler & Benz, 1987; Symondson et al., 2002; Hoefler et al., 2006). Although the use of spiders as BCAs has not yet been embraced on a global scale, there have been reports regarding its success in Southeast Asia. Growers in Japan, the Philippines and China have recognized the predatory significance of spiders in reducing pests in rice fields (Kiritani & Kakiya, 1975; IRRI, 1976; Kiritani, 1979; USDA, 1982; Riechert & Bishop, 1990). More recent studies by Thorbek et al. (2004) in Denmark and the U.K. found that linyphiid spider species would make good candidates as BCAs because of their early and continued reproductive activity throughout the growing season. Additionally, some jumping spiders, *Phiddipus* spp. (Araneae: Salticidae), were promoted as BCAs by Hoefler et al. (2006) in light of their proficient hunting and ease of rearing. Many of these characteristics parallel those of some current BCAs and thus may contribute to the future implementation of spiders as BCAs (Symondson et al., 2002; van Lenteren, 2012).
Predatory mites are arachnids that are increasingly used in augmentative biological control. The mite *Amblyseius swirskii* (Mesostigmata: Phytoseiidae), for example, is a generalist predator that has been widely used to control whiteflies, thrips, and herbivorous mites in greenhouses (Calvo & Belda, 2007; Calvo *et al*., 2011). Their popularity and success have been attributed to their polyphagous feeding behaviour, limited dispersal, and ease of rearing and release (Calvo *et al*., 2011). True spiders (Order: Araneae) possess similar behavioural characteristics, but rearing and release may be more difficult. Mechanical methods of release such as shakers and blowers would likely cause serious injury to true spiders, especially for larger species. Other less abrasive release methods have been suggested by Young and Edwards (1990), for example, the placement of field collected or laboratory reared egg sacs on crop vegetation. Augmentation of this kind might be most effective in greenhouse systems where assemblages of spiders may not be as diverse as those in the field (Provencher & Riechert, 1994). This further demonstrates the promise of spiders as BCAs, therefore, it is important that we understand the impacts of agricultural practices, especially pesticides, on them.

### 1.5 Toxicology and detoxification in spiders

Ecotoxicology investigates the effects of toxic chemicals on biological organisms at various levels of organization in an ecosystem (Truhaut, 1977; Stenersen, 2004). By thoroughly understanding the impact of toxicants on individual organisms, populations and communities, we can better appreciate the parameters and conditions required to conserve and maintain healthy and balanced ecosystems (Clements and Rohr, 2009). Toxicants present in the environment typically derive from either natural (*e.g.*, mycotoxins produced by fungi, various plant toxins, *etc.*) or man-made sources (*e.g.*, synthetic pesticides). Exposure by contact, inhalation, or ingestion of
toxicants from contaminated foods, substrates, air or water can be detrimental to the health of an organism and lead to death, developmental and reproductive complications, injury, disease, mutation, and other ill-effects (Stenersen, 2004; Relyea & Hoverman, 2006). At the population and community scale, consequences can include negative impacts on species abundance, richness, and interactions; with trophic exchange and buildup of toxicants occurring through bioconcentration, bioaccumulation, and biomagnification (Atwell et al., 1998; Landrum & Fisher, 1999; Gray, 2002). Studying the acute and chronic effects of toxicants on organisms and their interactions has been a major facet of ecotoxicology research because of its importance in characterizing our involvement and influence on the global food web. In particular, this includes concerns regarding risks and hazards to human health, our impact on the environment, and the corresponding repercussions for agricultural practice (Moriarty, 1988).

Spider ecotoxicology is a field of study still in its infancy with a great deal of knowledge remaining to be discovered. According to a database survey of natural enemies affected by pesticides, Theiling and Croft (1988) reported that only 3% of the toxicology papers they reviewed focused on spiders. This has surely increased since, 1988; however, even based on the limited data available, like other beneficial arthropods, spiders can be negatively affected by the use of pesticides (Symondson et al., 2002; Pekár, 2012, 2013; Desneaux et al., 2007). Consequently, the efficacy of strategies like IPM can be limited when growers employ pesticides that negatively impact beneficial arthropod communities. This often happens unknowingly because growers depend on pesticide manufacturers to develop products that are highly selective for target pests (i.e. products that will best adhere and mesh with IPM strategies by minimizing risk to non-target organisms). Despite this, pesticides that demonstrably have negative impacts on at least some beneficial arthropods continue to be released to market (e.g. neonicotinoids, pyrethroids, and more—See
review by Talebi et al., 2008). These compounds can impair the ability of beneficial organisms, including spiders, bees, parasitic wasps, and various predatory insects, from contributing integral ecosystem services (Symondson et al., 2002; Blacquière et al., 2012; Dempsey & Robertson, 2012; Pekár, 2012). Non-selective pesticides that hinder or remove natural enemies from the population can result in community instability, which can lead to the proliferation of primary and unexpected secondary pests (Settle et al., 1996; Symondson et al., 2002). Therefore, although pesticides are not designed with the intent of targeting beneficial arthropods, they can, nonetheless, be affected which can be counterproductive in IPM practice.

The ability of spiders to suppress pests has been shown to be negatively affected by the use of pesticides (Pekár, 2012, 2013). Spiders are subject to lethal, sublethal (e.g. interference with locomotion, predation, web-building, reproduction, and development), and indirect (e.g. herbicides decrease web attachment sites for web-spinning spiders) effects of pesticides (Pekár, 2012, 2013). A review conducted by Pekár (2012) collated toxicology research pertaining to spiders prior to 2012 and found that of 130 pesticides tested on about 50 spider species, acaricides and broad-spectrum insecticides proved to be the most detrimental. This includes compounds such as pyrethroids, organophosphates, carbamates and cyclodienes, most of which are neurotoxic excitatory agonists causing tetany, ataxia, and rigid paralysis. Starvation and/or dehydration result due to their immobilizing effects (Pekár, 2012). By studying the effects of pesticides on spiders and seeking out those which are most selective, we will better be able to identify and minimize the impacts of non-selective compounds on this significant group of natural enemies.

Spiders can sometimes overcome the toxic effects of pesticides and other xenobiotics by metabolic detoxification. A few studies have demonstrated that spiders employ glutathione S-
transferase (GST), glutathione peroxidase (GSH-Px), and carboxylesterases for the metabolism of toxicants (Nielsen et al., 1999; van Erp et al., 2002; Wang et al., 2006c). Studies by Hopkin (1990) found that the protein metallothionein and the ferritin metabolite haemosiderin play a role in the sequestration of heavy metals in the digestive tract cells of Dysdera crocata (Araneae: Dysderidae). Although these studies have identified some of the enzymes responsible for Phase-II detoxification and sequestration in spiders, they did not investigate the most ubiquitous metabolic enzyme system found in nearly every organism: Phase-I detoxification by cytochrome P450 enzymes (Yang et al., 2011). Cytochrome P450 has been well studied in other arthropods and has been found to be central in the metabolism of many xenobiotics, including pesticides (Li et al., 2007b; Gilbert, 2009). The occurrence of P450 enzymes in spiders is known from sequence analyses conducted by Yang et al. (2011) on Ummeliata insecticeps (Araneae: Linyphiidae). However, the ability of cytochrome P450 to protect spiders from pesticides has not been investigated. One approach to demonstrate the role of P450 is to conduct inhibition assays by co-applying insecticides with piperonyl butoxide (PBO), a methylenedioxyphenyl synergist commonly used in combination with natural pyrethrins and synthetic pyrethroids to enhance their efficacy (Casida, 1970; Correia & de Montellano, 2005). PBO binds to cytochrome P450 with high affinity creating a complex that prevents further binding of P450 to xenobiotics (Casida, 1970; Correia & Montellano, 2005). This compromises oxidative detoxification and increases the duration of the toxicant within the insect.

1.6 Diamide Insecticides

Diamides insecticides have a specific target site and novel mode of action previously unexploited by other commercial insecticides (Satelle et al., 2008—See Appendix 5 for the chemical structures of diamide insecticides and the physicochemical properties of chlorantraniliprole). Most
insecticides are neurotoxic and target neuronal cells; in contrast diamides are muscle toxins. Diamides bind to and modulate ryanodine receptors (RyRs), non-voltage-gated transmembrane calcium channels responsible for mediating calcium-induced calcium release from intracellular stores. Ryanodine, a natural alkaloid derived from the tropical plant *Ryania speciosa* (Malpighiales: Salicaceae), binds to these channels and has played a fundamental role in their discovery and characterization (Jenden & Fairhurst, 1969; Fill & Copelo, 2002). Ryanodine receptors occur in the sarcoplasmic reticulum of muscle cells and the endoplasmic reticulum of many other cell types in animals (Satelle et al., 2008). Their homotetrameric structure consists of four identical subunits each with a large N-terminal region extending into the cytosol and a C-terminal region comprising the ion-conducting pore (Ogawa & Murayama, 1998; Xu et al., 2000; Hamilton, 2005; Lanner et al., 2010). Regulation of cytoplasmic concentrations of calcium by selective release from intracellular stores is crucial for a number of cell functions, including muscle contraction, neurotransmission, hormone release, gene expression, growth and differentiation (Hamaguchi et al., 2012). Diamides cause prolonged activation of RyRs in insect muscle cells resulting in excessive and uncontrolled calcium release and depletion of intracellular calcium stores (Lahm et al., 2005; Cordova et al., 2006; Satelle et al., 2008). In muscle cells the resulting flood of calcium into the cytosol results in gradual and sustained contraction leading to the rapid cessation of feeding, rigid paralysis, and ultimately, death (Cordova et al., 2006).

The diamide insecticides have high affinity for RyRs in insect muscles (Hirooka et al., 2007; Lahm et al., 2007a; Lahm et al., 2009; Selby et al., 2013). Many lepidopteran species are susceptible to ryanoids. In addition, various beetles (Coleoptera: Chrysomelidae; Curculionidae; Scarabaeidae), leafminers (Diptera: Agromyzidae), whiteflies/leafhoppers (Hemiptera: Aleyrodidae; Cicadellidae), thrips (Order: Thysanoptera), and termites (Order: Blattodea) are effectively controlled by diamides.
The translaminar movement and translocative properties of this class of compounds in crop plants makes them particularly effective at protecting the entire plant against chewing, sucking, boring, and rasping pests for up to 28 days after application (DuPont Technical Bulletin, 2008). Although RyRs are ubiquitous in the muscle, neuronal, and epithelial cells of many organisms, the diamide insecticides are generally selective for arthropod RyRs (Satelle et al., 2008) and have very low mammalian toxicity, with an acute oral LD$_{50}$ of >5000 mg kg$^{-1}$ in rats (for chlorantraniliprole) (Lahm et al., 2007a). Chlorantraniliprole has between 300 and 2000-fold selectivity for insect RyRs over mammalian RyRs (Lahm et al., 2007a). Intrinsic structural differences in RyRs, likely as a result of genetic diversification (Waterhouse et al., 1987; Jeffries et al., 1997; Satelle et al., 2008), are probably the basis for the high selectivity of these compounds (Xu et al., 2000; Cordova et al., 2006; Lahm et al., 2007a; Hamaguchi et al., 2012). Extensive research on RyRs in mammals has revealed the existence of three isoforms specific to various muscle types (Marks et al., 1989; Bennett et al., 1996; Fill & Copello, 2002). Insects in contrast possess only a single isoform (Takeshima et al., 1994; Lahm et al., 2009). Based on their negligible mammalian toxicity the ryanoids have been marketed as low-risk insecticides suitable for rotation in IPM strategies (Dinter et al., 2008; DuPont Technical Bulletin, 2008). However, the selectivity of diamides among arthropods other than insects, especially for non-target arthropods such as spiders, remains relatively unknown.

Coragen and Altacor®, both of which contain Rynaxypyr® (active ingredient = chlorantraniliprole) as their active ingredient, were introduced in North America as broad-spectrum insecticides by DuPont in 2008. Coragen is registered for use in a wide variety of crops including brassica, cucurbit, fruiting, leafy, legume, tuberous and corm vegetables as well as corn, canola, cereals, potatoes, sunflowers, okra, mint, grass forage, and non-grass forage (alfalfa, clover and
lupin) at field rates of 50–100 g a.i. ha\(^{-1}\) (DuPont Technical Bulletin, 2008). Altacor is registered for use in stone fruit, pome fruit, grapes, tree nuts, and various berry groups at rates of 145–285 g a.i. ha\(^{-1}\) (DuPont Altacor Main Label, 2013). Rates as low as 1–2 g a.i. ha\(^{-1}\) have been used for *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) and rates as high as 230 g a.i. ha\(^{-1}\) for scarab grub control (Coleoptera: Scarabaeidae) (Lahm *et al.*, 2007b; Larson *et al.*, 2012). At these application rates these compounds are reported to have limited activity on some beneficial non-target arthropods. Laboratory and field studies have shown low risk and little impact on populations of parasitic wasps, predatory mites, predatory insects (*e.g.* *Orius* spp. and *Nabis* spp.), green lacewings, and lady beetles (Dinter *et al.*, 2008; Preetha *et al.*, 2009; Brugger *et al.*, 2010; Gradish *et al.*, 2011). The bumble bee, *Bombus impatiens* (Hymenoptera: Apidae), is unaffected by chlorantraniliprole exposure at rates of 0.01, 0.1, and 1.0 g L\(^{-1}\) (Gradish *et al.*, 2010). However, these findings encompass only a small proportion of the beneficial non-target arthropod species present in field crops. Further studies need to be conducted on other non-target predatory arthropods such as spiders to broaden our understanding of the potential impacts of newly introduced insecticides in crop systems.

### 1.7 Research Objectives

In this thesis, I explore the lethal and sublethal effects of a recently introduced anthranilic diamide (ryanoid) insecticide, Coragen (product of DuPont), on two crop-representative spiders, *Enoplognatha ovata* and *Tibellus* spp. Additionally, the enzyme inhibitor piperonyl butoxide (PBO) was used in assays to investigate the potential detoxification pathways responsible for metabolizing Coragen in these spiders. Lethal effects were investigated through the direct topical application of Coragen to spiders using a miniature spray tower. Concentrations were applied at and below tank
concentrations comparable to those recommended by DuPont. The rates of application per unit area were at or below those recommended for field use. Sublethal effects were investigated through monitoring feeding behaviour at low, non-lethal dosages. This was quantified as the rate of predation on fruit flies by spiders after spraying with Coragen. Lastly, I compare and discuss the findings for both spider species.

Summary of Research Objectives:

1. Determine the toxicity of topical applications of Coragen on adult female *Enoplognatha ovata* and *Tibellus* spp.

2. Evaluate the sublethal effects of Coragen on predation by *Enoplognatha ovata* and *Tibellus oblongus*, and *Tibellus maritimus*.

3. Determine if the toxic effects of topical applications of Coragen are synergized by piperonyl butoxide (PBO). Determine potential detoxification pathways for Coragen metabolism.

Summary of Null Hypotheses:

1. DuPont claims Coragen is harmless to a variety of beneficial arthropods, including predatory mites. Due to physiological similarities, it was expected that Coragen would be harmless to spiders as well.

2. For similar reasons, it was expected that sublethal concentrations of Coragen would not negatively impact predatory behaviour in spiders.

3. Co-application of the synergist piperonly butoxide (PBO) was not expectedto affect susceptibility of spiders to Coragen.
2.1 Introduction

In this study, I determined the susceptibility of a common field spider, *Enoplognatha ovata* (Clerck, 1757), to the recently registered and commercially available diamide insecticide, Coragen. The need to address pesticide resistance and non-target toxicity continues to drive the discovery and development of compounds with novel chemistries, target sites, and modes of action. Diamides were released in North America in 2008 and have been assigned to the new IRAC Group 28 in view of their novel mode of action. There are two groups of diamide insecticides: pthalic diamides, including flubendiamide, and anthranilic diamides, including chlorantraniliprole and cyantraniliprole (Tohnishi *et al.*, 2005; Lahm *et al.*, 2007a; Selby *et al.*, 2013). Flubendiamide was first released in 2006 in the Philippines. Chlorantraniliprole was introduced in 2007 in Thailand. Both diamides were initially released to control lepidopterous pests (Tohnishi *et al.*, 2005; Lahm *et al.*, 2007a). Since their introduction, they have gained considerable momentum in the global market with sales and registration in over 80 countries (Troczka *et al.*, 2012) and gross revenues projected to exceed $2B by 2020 (Teixeira & Andaloro, 2013). The growing popularity and use of diamide insecticides may put them at risk of overuse if not managed and rotated properly. This could result in the development of resistance and unforeseen deleterious effects on non-target organisms. Thoroughly researching these newly introduced compounds and understanding their impacts on agroecosystems is vital to prolonging their effective use.
Spiders are an important group of natural enemies ubiquitous in agroecosystems. Owing to their wide distribution and diversity, spiders comprise one of the most abundant groups of natural enemies in agrobiocenoses (Nyffeler & Benz, 1987; Sterling et al., 1992; Zhang, 1992; Nyffeler & Sunderland, 2003; Ludy & Lang, 2004). These generalist predators have demonstrated the ability to suppress pest populations in a variety of crop systems, including soybean (Carter & Rypstra, 1995), apple and citrus orchards (Mansour et al., 1980; Mansour & Whitcomb, 1986), rice (Itô et al., 1962; Oraze & Grigarick, 1989), taro (Nakasuji et al., 1973), cotton (Mansour, 1987a), spring barley (Chiverton, 1986), sweet basil (Hoefler et al., 2006), and vegetables (Riechert & Bishop, 1990). Spiders preying on pest populations can contribute to a substantial reduction in crop damage resulting in increased yields and profits for growers (Riechert & Bishop, 1990; Carter & Rypstra, 1995; Sunderland, 1999; Hlivko & Rypstra, 2003).

The ability of spiders to suppress pests can be adversely affected by exposure to insecticides (Pekár, 2012, 2013). Insecticides can impact spiders both directly (i.e. lethal and sublethal effects) and indirectly (i.e. changes to environment or prey availability). In a review by Pekár (2012), acaricides and broad-spectrum insecticides were found to cause significantly higher mortality to spiders than herbicides and fungicides. In particular, organophosphates, carbamates, pyrethroids, and cyclodiene were the most potent neurotoxic compounds to spiders in acute toxicity tests (Pekár, 2012). In addition to direct lethal effects, sublethal concentrations of insecticides can interfere with spider locomotion, predation, web-building, reproduction, and development (Pekár, 2013). Sublethal effects should not be overlooked in toxicity studies as they can be very debilitating for pest suppression by spiders (Pekár, 2012, 2013). Spiders can also be affected by changes to the environment, including the reduction of vegetation due to herbicide applications, which can limit web attachment sites or reduce available prey (Haughton et al., 1999; Markó et al., 2009). It is
especially critical to understand the lethal and sublethal activity of newly introduced insecticides like Coragen with a novel mode of action and with relatively little prior testing on spider toxicity (Rajavel et al., 2011; Yang et al., 2013).

The common candy-striped spider, *Enoplognatha ovata*, is a member of the comb-footed spider family, Theridiidae (Figure 2.1). This species is native to Eurasia, but has been widely introduced globally, including North America (Bristowe, 1941; Oxford & Reillo, 1994; Marusik & Koponen, 2005). These spiders can be locally very abundant. At some collection sites in southern Ontario, Canada, populations exceeded 50 spiders m\(^{-2}\) (pers. observation). Studies by Oxford and Shaw (1986) found densities in North Yorkshire, England reaching upwards of 18 spiders m\(^{-2}\).

*Enoplognatha ovata* occurs in weedy, herbaceous habitats, including the understory of woodlands, meadows, and fields (Bristowe, 1958; Reillo & Wise, 1988; Brierton et al., 2003). They are often associated with crop margins where they overwinter in leaf litter (Oxford & Shaw, 1986). They migrate into fields from margins when seeking food, mating opportunities, and nesting sites (Barbosa, 1998; Landis et al., 2000; Opatovsky et al., 2012). Their presence in crops and field margins could potentially result in exposure to Coragen through direct topical application, contact with residues, or drift into adjoining field margins. Although *E. ovata* have been studied extensively with respect to colour polymorphism (e.g., genetic, biochemical, and frequency), ontogeny, and general ecology (Bristowe, 1958; Seligy, 1971; Oxford, 1983, 2005; Oxford & Shaw, 1986; Reillo & Wise, 1988), there has been little research conducted on their susceptibility to insecticides (Zimakowska-Gnoinska & Tarwid, 1984).
In this study, the effects of Coragen on *E. ovata* were studied. In the first experiment, the lethal concentration for adult female *E. ovata* was determined. In the second experiment, the effects of sublethal exposure were investigated by evaluating impact on predation. These were quantified as the rate of spider predation on *Drosophila* spp. in arena tests. In the third experiment, the synergistic effect of PBO with Coragen was evaluated to identify potential detoxification pathways responsible for Coragen metabolism in *E. ovata*.

Figure 2.1. An adult female candy-striped spider (*Enoplognatha ovata*). Body length: 4–7 mm. Photos: Dr. Jonathan Schmidt.
2.2 Materials and Methods

2.2.1 Spider collecting and rearing

*Enoplognatha ovata* (Araneae: Theridiidae) were collected from annual sow thistle, *Sonchus asper* (L.) Hill (Asterales: Asteraceae), at a semi naturalized meadow site in Elora, Ontario, Canada (43.67338N, 80.42351W) during June and July of 2013 and 2014. Late instar and adult female spiders dominated the field populations sampled in both years. Spiders were captured by hand and placed into either 1.5 mL microcentrifuge tubes or 15 mL conical centrifuge tubes (Fisher Scientific, Ottawa, Canada). Spiders were later transferred to 125 mL Bernardin® Mason jars where they were individually housed and fed until used in experiments. The jars were capped with mesh lids for ventilation and ease of watering. Each jar contained 1–2 small sticks as substrates to accommodate the various hunting strategies employed by *E. ovata* (web-building, sit and wait ambush, and cursorial hunting) (Bristowe, 1941; Agnarsson, 2002). Deionized water was applied weekly to the mesh lids of the containers using a spray bottle. The spiders were fed two fruit flies (*Drosophila* spp. (Diptera: Drosophilidae)) each week (See Appendix 1: Fruit Fly Rearing). Feeding usually coincided with watering. All spiders used in toxicity tests were starved for one week before experiments to standardize satiation. During the 2013 field season, spiders were kept in a growth room with controlled temperature and humidity (17 ± 1°C, 60 ± 8% RH). The cool temperature of the growth room was intended to slow the metabolism and energy demands of the spiders, and to prolong their longevity (Foelix, 2010). High ambient humidity was essential to mitigate dehydration and desiccation. During the 2014 field season, spiders were maintained at ambient lab conditions (21.5 ± 1°C, 50 ±8 % RH). There were no differences in the mortality of spiders in control treatments during the course of testing between years (i.e., 0% in both years). Temperature and humidity
measurements were taken using a Traceable™ thermometer/clock/humidity monitor (Fisher Scientific, Ottawa, Canada).

2.2.2 Spider selection for testing

Prior to experimentation, spiders were sorted based on gender and life stage. Gender was determined by examining the pedipalps of the spiders with the naked eye. Females have smooth cylindrical distal tarsi, whereas males have clubbed or bulbous distal tarsi (Seligy, 1971; Foelix, 2010; See Appendix 4: Sexing Spiders by Pedipalps). Life stage for each spider was determined using body size and genital development parameters outlined by Seligy (1971). Only adult female spiders were used for toxicity testing. Female spiders are generally larger than males or juveniles and have been reported to have a higher tolerance to pesticides (Deng et al., 2006; Dinter & Poehling, 1995). Male E. ovata are relatively short-lived in the field and would have higher background mortality in toxicity tests than adult females (Seligy, 1971; Pekár, 2012). Spiders were randomly assigned to treatment groups in all experiments. This was accomplished by assigning each spider a unique random number using the random number generator of a calculator.

2.2.3 Chemicals tested

Spray formulations were prepared from a stock solution of 200 g L\(^{-1}\) Coragen obtained from DuPont Canada (Mississauga, Ontario, Canada). Dilutions were prepared with deionized water to obtain selected concentrations. The stock solution contained a guaranteed active ingredient concentration of 18.4% chlorantraniliprole (CAS # = 500008-45-7) (3-Bromo-N-[4-chloro-2methyl-6-[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide). The insecticide synergist piperonyl butoxide (PBO) (CAS # = 51-03-6) (5-[2(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole) was obtained from Acros Organics (New
Jersey, USA). Formulations were prepared from a stock solution of 90% PBO through a two-phase dilution series. One mL of 90% PBO was initially diluted with 95% ethanol (polar protic solvent) to a total volume of 10 mL. This was followed by a second dilution with deionized water to a total volume of 90 mL to obtain a 1% PBO solution. Fresh formulations were prepared before each test.

2.2.4 Spray apparatus and application

Coragen, PBO, and water were applied topically to spiders as aerosols using a miniature spray tower. The spray tower consisted of a metal tube with an Iwata® Eclipse HP-BCS airbrush (Wyndham Art Supplies, Guelph, Canada) centred at the top of it (Gradish et al., 2009a, 2009b). An air compressor delivering 15 PSI powered the apparatus. The spray pattern was tested and calibrated by counting droplets on water-sensitive paper (Syngenta, Basel, Switzerland) for a 1 mL volume of deionized water. The sprayer delivered a conical spray pattern that evenly stained the test paper with a distribution of about 30–50 droplets cm$^{-2}$. Further testing of the spray tower was conducted to determine the actual deposition rate for a 1 mL load of solution (See Appendix 2: Spray Tower Calibration). Based on these measurements, it was determined that 0.0214 ± 0.0009 mL of solution were deposited over the target area (28.274 cm$^2$) during each application.

Spiders were placed individually into 60 × 15 mm Pyrex® Petri dishes (Fisher Scientific, Ottawa, Canada) prior to being sprayed. Each dish was lined with a 4.7 cm diameter Whatman #5 filter paper (Fisher Scientific, Ottawa, Canada) as a substrate. Prior to spraying, spiders were placed in a freezer at -18 ± 1°C for 5 min to immobilize them during spraying. Freezing and spraying typically occurred in groups of 5 to ensure that the spiders did not become active prior to treatment. For each treatment the spray tower was loaded with 1 mL of either water, Coragen, or
PBO using an Oxford® BenchMate® II single-channel, piston-driven, air-displacement pipette with disposable tips (Nichiyto, Maryland Heights, USA). The spray tower was cleansed with acetone and deionized water between treatments to eliminate the potential for cross-contamination. All tests were isovolumetric and used 1 mL of solution to ensure similar saturation of the test dish surface. A volume of 1 mL was selected as higher volumes caused pooling on the filter paper.

2.2.5 Experiment 1: Dose-response testing

Coragen was tested at concentrations ranging from 0.028 to 1.125 mg mL\(^{-1}\) which corresponded to corrected application rates of 2.13 to 85.15 g ha\(^{-1}\) (Table 2.1). These ranges included the recommended tank mixture concentrations (0.5–1.0 mg mL\(^{-1}\)) and were similar to the recommended field application rates (50–100 g ha\(^{-1}\)) (DuPont Supplemental Label, 2010). The number of spiders tested at each treatment level was 16 in 2013 and 20 in 2014. Deionized water was used as a negative control in each test series. The mortality of spiders was assessed by checking for responsiveness at 1, 24, and 48 h after spraying. A small, metal probe was used to poke the dorsal side of the abdomen to elicit a response. Knockdown (paralysis) was expected to occur within minutes to a few hours after spraying (DuPont Technical Bulletin, 2008; Bassi et al., 2009; Hamaguchi and Hirooka, 2012). Following the 48 h check, unresponsive spiders were considered to be dead. Spiders were preserved in 95% ethanol for future reference or measurements.
Table 2.1. Tested concentrations and corresponding application rates of Coragen (active ingredient = chlorantraniliprole; DuPont).

<table>
<thead>
<tr>
<th>Concentrations tested (mg/mL)</th>
<th>Corrected rates (μg/cm²)ᵃ</th>
<th>Equivalent field rates (g/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.028</td>
<td>0.021</td>
<td>2.13</td>
</tr>
<tr>
<td>0.056</td>
<td>0.043</td>
<td>4.26</td>
</tr>
<tr>
<td>0.113</td>
<td>0.085</td>
<td>8.51</td>
</tr>
<tr>
<td>0.225</td>
<td>0.170</td>
<td>17.03</td>
</tr>
<tr>
<td>0.281</td>
<td>0.213</td>
<td>21.29</td>
</tr>
<tr>
<td>0.338</td>
<td>0.255</td>
<td>25.54</td>
</tr>
<tr>
<td>0.450</td>
<td>0.341</td>
<td>34.06</td>
</tr>
<tr>
<td>0.563ᵇ</td>
<td>0.426</td>
<td>42.57</td>
</tr>
<tr>
<td>1.125ᵇ</td>
<td>0.852</td>
<td>85.15</td>
</tr>
</tbody>
</table>

ᵃAccounting for area of test dish (A = 28.274 cm²) and 2.14% spray tower correction factor (Appendix 3).

ᵇLast two rows represent concentrations and tank mix rates similar to those recommended by DuPont.

2.2.6 Experiment 2: Testing sublethal effects of Coragen on predation

Spiders were treated as described in experiment-1 with either 0.028 mg mL⁻¹ or 0.056 mg mL⁻¹ of Coragen. These concentrations were selected because they resulted in less than 50% mortality in experiment-1. Control spiders were treated with deionized water in each test series. Spiders were left for 2 h in the initial test dishes, only spiders that were responsive after this time were used for feeding trials. Spiders were subsequently transferred to larger 100 × 25 mm plastic Petri dishes (Fisher Scientific, Ottawa, Canada) which contained a piece of paper towel for substrate and a cotton swab saturated with deionized water for moisture. The use of different dishes ensured that the fruit flies would not be exposed to Coragen residues. For the feeding tests, each spider was provided with 10 fruit flies. Dishes containing only fruit flies were also prepared to monitor the background mortality of fruit flies. Fly mortality was checked at 4 and 24 h after they had been provided to the spiders. An initial check after 4 h was chosen because *E. ovata* feed primarily within the first few hours after being introduced to prey (pers. observation). The 24 h check was to monitor the status of the treated spiders. The mortality of spiders was assessed as in...
experiment-1 and only data for responsive spiders was included in the analysis. Spiders were preserved in 95% ethanol following completion of the experiment.

2.2.7 Experiment 3: Testing the synergistic effects of PBO

Four treatments were tested: water, 1% PBO alone, Coragen alone, and 1% PBO and Coragen in combination. Coragen was applied at two different concentrations: 0.056 mg mL\(^{-1}\) in trial-1 and 0.028 mg mL\(^{-1}\) in trial-2. The concentration of PBO tested in both trials was 1% (10 mg mL\(^{-1}\)). This concentration of PBO was selected based on the high end of typical co-application rates with pyrethrin and pyrethroid insecticides. In preliminary tests, no mortality was observed after 48 h with 2% PBO. The combined application of PBO and Coragen occurred sequentially. Spiders were initially divided into two groups and sprayed with either deionized water or 1% PBO. Two hours later, each of those groups were divided again and sprayed with deionized water and Coragen. This sequential two stage spraying method allowed me to test every permutation of water, PBO, and Coragen (i.e., water-water, water-Coragen, 1% PBO-water, and 1% PBO-Coragen). It also allowed ample time for PBO to take effect. Ten spiders were tested at each treatment level. Assessing the mortality of spiders and the preservation of dead spiders proceeded as in the previous experiments.

2.2.8 Statistical analysis

For experiment-1, treatment concentrations were converted using a log transformation in order to acquire a linear relationship between Probit mortality and concentration. A Probit regression analysis was then conducted to calculate the LC\(_{50}\) value with corresponding 95% confidence intervals. A Chi-square test was used to determine the goodness-of-fit of the observed values to the predicted values in the Probit regression. In experiment-2, a nonparametric Mann Whitney U test was used to compare a continuous outcome (mortality) with two independent
samples (Coragen treated and untreated spiders). A nonparametric Mann-Whitney U test was selected instead of a standard t-test for independent samples because sample sizes were smaller than 30 and normality could not be assumed. The Mann-Whitney U test assumes homogeneity of variance, and this was tested for each treatment using a nonparametric Levene’s test. IBM SPSS Ver. 22.0 (IBM Corp., Armonk, NY) was used to perform all statistical computations. A Type I error of 0.05 was used for all statistical tests.

2.3 Results

2.3.1 General observations of the effects of Coragen

*Enoplognatha ovata* were observed over a period of 48 h after being sprayed with Coragen or water. Both Coragen- and water-treated spiders exhibited tarsal grooming which lasted approximately 30–50 min, following exposure. After this initial bout of activity, both Coragen and water treated spiders displayed little or no subsequent movement unless disturbed with a metal probe. After 24 h, spiders treated with higher concentrations of Coragen did not react when disturbed and instead maintained a rigid posture with all legs outstretched.

2.3.2 Experiment 1: Dose-response testing

Over the wider range of concentrations tested in 2013 and 2014, mortality in adult female *E. ovata* increased with increasing concentrations of Coragen (Table 2.3, 2.4). In preliminary range finding tests, mortality was 12% at 0.056 mg mL\(^{-1}\), 94% at 0.563 mg mL\(^{-1}\), and 90% at 1.125 mg mL\(^{-1}\) after 24 h. Control mortality was 0% after 48 h in all trials. Mortality increased between each check of spider responsiveness and reached a peak at 24 h. This suggests that it takes several hours for Coragen to take effect in adult female *E. ovata*, even at higher concentrations. In definitive tests,
after 48 h, Coragen tested at 0.563 mg mL\(^{-1}\) resulted in 80% mortality in 2013 and 70% mortality in 2014 (Table 2.3, 2.4). According to standards set by the International Organization of Biological Control (IOBC), these findings indicate that Coragen is moderately harmful (51–75% mortality) to harmful (>75% mortality) to adult female *E. ovata* at rates comparable to those recommended for field application (Hassan, 1985). The extrapolated field rate for 0.563 mg mL\(^{-1}\) is 42.57 g ha\(^{-1}\) (Table 2.1), slightly below the lowest field rate recommended by DuPont, 50 g ha\(^{-1}\) (DuPont Supplemental Label, 2010). The results demonstrate that the RyRs of *E. ovata* are susceptible to Coragen at concentrations similar to those recommended for use against many target insect pest species (DuPont Technical Bulletin, 2008; DuPont Supplemental Label, 2010).

The log-probit LC\(_{50}\) value was 0.210 mg mL\(^{-1}\) with a 95% confidence interval of 0.161 to 0.279 mg mL\(^{-1}\). This included mortality data from both 2013 and 2014. The log-probit model displayed adequate fit for the relationship between dosage concentrations and observed and fitted probabilities with a Pearson Goodness-of-Fit p-value of 0.179 (Table 2.2).

<table>
<thead>
<tr>
<th>Conc. (mg/mL)</th>
<th>Log of Conc.</th>
<th>Number of spiders tested</th>
<th>Total observed mortality</th>
<th>Total expected mortality</th>
<th>Residual</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0.000</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.028</td>
<td>-1.551</td>
<td>16</td>
<td>2</td>
<td>0.919</td>
<td>1.081</td>
<td>0.057</td>
</tr>
<tr>
<td>0.028</td>
<td>-1.551</td>
<td>20</td>
<td>0</td>
<td>1.148</td>
<td>-1.148</td>
<td>0.057</td>
</tr>
<tr>
<td>0.056</td>
<td>-1.249</td>
<td>10</td>
<td>3</td>
<td>1.509</td>
<td>1.491</td>
<td>0.151</td>
</tr>
<tr>
<td>0.113</td>
<td>-0.949</td>
<td>16</td>
<td>5</td>
<td>4.992</td>
<td>0.008</td>
<td>0.312</td>
</tr>
<tr>
<td>0.113</td>
<td>-0.949</td>
<td>20</td>
<td>5</td>
<td>6.240</td>
<td>-1.240</td>
<td>0.312</td>
</tr>
<tr>
<td>0.225</td>
<td>-0.648</td>
<td>16</td>
<td>7</td>
<td>8.337</td>
<td>-1.337</td>
<td>0.521</td>
</tr>
<tr>
<td>0.281</td>
<td>-0.551</td>
<td>20</td>
<td>10</td>
<td>11.802</td>
<td>-1.802</td>
<td>0.590</td>
</tr>
<tr>
<td>0.338</td>
<td>-0.472</td>
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<td>10</td>
<td>10.312</td>
<td>-0.312</td>
<td>0.644</td>
</tr>
<tr>
<td>0.450</td>
<td>-0.347</td>
<td>16</td>
<td>16</td>
<td>11.590</td>
<td>4.410</td>
<td>0.724</td>
</tr>
<tr>
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<td>8</td>
<td>7.796</td>
<td>0.204</td>
<td>0.780</td>
</tr>
<tr>
<td>0.563</td>
<td>-0.250</td>
<td>20</td>
<td>14</td>
<td>15.591</td>
<td>-1.591</td>
<td>0.780</td>
</tr>
</tbody>
</table>

(Total number of spiders tested = 216)
Table 2.3. Summary of dose-response data for adult female *Enoplognatha ovata* exposed to Coragen (active ingredient = chlorantraniliprole) applications in 2013.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>No. of Spiders Tested</th>
<th>% Mortality (1-h after spraying)</th>
<th>% Mortality (24-h after spraying)</th>
<th>% Mortality (48-h after spraying)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>16</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.028</td>
<td>16</td>
<td>6.3</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>0.056</td>
<td>10</td>
<td>20.0</td>
<td>30.0</td>
<td>30.0</td>
</tr>
<tr>
<td>0.113</td>
<td>16</td>
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<td>31.3</td>
</tr>
<tr>
<td>0.225</td>
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<td>25.0</td>
<td>43.8</td>
<td>43.8</td>
</tr>
<tr>
<td>0.338</td>
<td>16</td>
<td>43.8</td>
<td>62.5</td>
<td>62.5</td>
</tr>
<tr>
<td>0.450</td>
<td>10</td>
<td>50.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>0.563</td>
<td>10</td>
<td>30.0</td>
<td>80.0</td>
<td>80.0</td>
</tr>
</tbody>
</table>

(Total number of spiders tested = 116)

Table 2.4. Summary of dose-response data for adult female *Enoplognatha ovata* exposed to Coragen (active ingredient = chlorantraniliprole) applications in 2014.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>No. of Spiders Tested</th>
<th>% Mortality (1-h after spraying)</th>
<th>% Mortality (24-h after spraying)</th>
<th>% Mortality (48-h after spraying)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.028</td>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.113</td>
<td>20</td>
<td>5.0</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>0.281</td>
<td>20</td>
<td>30.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>0.563</td>
<td>20</td>
<td>55.0</td>
<td>70.0</td>
<td>70.0</td>
</tr>
</tbody>
</table>

(Total number of spiders tested = 100)

2.3.3 Experiment 2: Testing sublethal effects of Coragen on predation

Very few fruit flies (<2%) were dead after 24 h in dishes that did not contain spiders, indicating that most or all of the observed fruit fly mortality can be attributed to predation by spiders in the treatments. In sublethal trial-1, no fruit fly deaths were observed at 24 h in dishes without spiders. In sublethal trial-2, a total of 6 fruit flies were found dead at 24 h in dishes without spiders (*i.e.*, 0.4 fruit flies dead per dish). Background fruit fly mortality was corrected with Abbott's formula in sublethal trial-2.
Sublethal applications of Coragen at concentrations of 0.056 mg mL\(^{-1}\) (sublethal trial-1) and 0.028 mg mL\(^{-1}\) (sublethal trial-2) had little effect on *E. ovata* predation on *Drosophila* spp. (Figure 2.2). In both sublethal trials, the average number of dead fruit flies was similar in dishes containing treated and untreated spiders after 4 and 24 h. For both trials, slightly more fruit flies were killed by Coragen treated spiders than untreated spiders. This was evident after 4 h of hunting time. The mean ranks for untreated spiders were lower than the mean ranks for Coragen treated spiders at both 4 and 24 h in each trial, but these differences were not found to be statistically significant using a Mann-Whitney U test (\(p>0.05\)). A nonparametric Levene’s test was used to verify the equality of variances in the samples (homogeneity of variance) (\(p>0.05\)) (Nordstokke & Zumbo, 2010; Nordstokke et al., 2011).

The mortality of spiders was comparable to those observed in experiment-1 for the same exposure levels. In sublethal trial-1, the mortality of spiders was 47% after treatment with Coragen at a concentration of 0.056 mg mL\(^{-1}\), comparable to the 30% mortality observed at the same concentration in experiment-1 for 2013. In sublethal trial-2, the mortality of spiders was 13% after treatment with Coragen at a concentration of 0.028 mg mL\(^{-1}\), comparable to the 13% mortality observed at the same concentration in experiment-1 for 2013. All control spiders, with the exception of a single individual in sublethal trial-1, survived treatment with deionized water.
Figure 2.2. Average number of fruit flies dead per dish after 4 and 24 h of allotted feeding time for adult female *Enoplognatha ovata*. In the top graph, spiders were treated with 0.056 mg mL\(^{-1}\) Coragen (Sublethal Trial-1). In the bottom graph, spiders were treated with 0.028 mg mL\(^{-1}\) Coragen (Sublethal Trial-2). Untreated spiders were sprayed with deionized water. Only responsive spiders were included. Error bars represent standard error of the mean.
2.3.4 Experiment 3: Testing the synergistic effects of PBO

In the two trials conducted during 2014, mortality 48 h after treatment was highest for spiders treated with a combination of Coragen (either 0.056 or 0.028 mg mL$^{-1}$) and 1% PBO compared to those treated with either 1% PBO (≤20%) or Coragen alone (0%) (Figure 2.3). No mortality was observed in the control (water-treated) groups. Mortality caused by Coragen alone was comparable to that observed in experiment-1. In a preliminary test combining a lower concentration of PBO (0.5%) and 0.056 mg mL$^{-1}$ Coragen, mortality was 80% after 48 h.

![Figure 2.3. Mortality of adult female Enoplognatha ovata 48 h after being treated sequentially with deionized water or 1% PBO, followed by an additional treatment with deionized water or Coragen. In Trial-1 (white bars), Coragen was applied at a concentration of 0.056 mg mL$^{-1}$. In Trial-2 (cross hatched bars), Coragen was applied at a concentration of 0.028 mg mL$^{-1}$.]
2.4 Discussion

2.4.1 The effects of diamides on spiders

Adult female *E. ovata* are susceptible to topical applications of Coragen at rates comparable to the recommended tank mix (0.5–1.0 mg mL\(^{-1}\)) and field application rates (50–100 g ha\(^{-1}\)) provided by DuPont (DuPont Technical Bulletin, 2008; DuPont Supplemental Label, 2010) (Table 2.1). In preliminary tests, Coragen at a rate of 1.125 mg mL\(^{-1}\) (or 85.15 g ha\(^{-1}\)) resulted in 90% mortality of spiders \((n=10)\) after 48 h. A rate of 0.563 mg mL\(^{-1}\) (or 42.57 g ha\(^{-1}\)) resulted in mortalities of 80% in 2013 \((n=10)\) and 70% in 2014 \((n=20)\) after 48 h (Table 2.3, 2.4). These mortalities would categorize Coragen as moderately harmful to harmful according to IOBC standards (Hassan, 1985). The behavioural responses to Coragen were also consistent with those observed for susceptible insects \((i.e.,\) rigid posture with all legs outstretched\) (Lahm *et al*., 2005; Cordova *et al*., 2006; DuPont Technical Bulletin, 2008; Satelle *et al*., 2008; Bassi *et al*., 2009; Hamaguchi and Hirooka, 2012). These findings suggest that Coragen acts with the same mode of action and at the same target site in *E. ovata* as it does in target insect pests. Differences between the RyRs of the target insect pests and *E. ovata* appear to be insufficient to result in selectivity.

The estimated LC\(_{50}\) for adult female *E. ovata* was approximately 210 mg L\(^{-1}\) with a 95% confidence interval of 161 to 279 mg L\(^{-1}\). This is comparable to the EC\(_{30}\) values observed in lab studies of flubendiamide of >100 mg L\(^{-1}\) for *Pardosa pseudoannulata* (Araneae: Lycosidae) and >200 mg L\(^{-1}\) for *Misumenops tricuspidatus* (Araneae: Thomisidae) (Lahm *et al*., 2007b). Susceptibility of spiders in the field has also been shown by Yang *et al.* (2013) who found as much as a 33% decrease (55.7 to 37.3 spiders/50 plants) in *Xysticus ephippiatus* (Araneae: Thomisidae) populations in maize fields three days following application with chlorantraniliprole at 48.1 mg L\(^{-1}\). These
populations rebounded after 9 days. However, several field studies have shown no effects attributable to diamide insecticides on spider populations. Studies in India by Rajavel et al. (2011) found no significant difference between spider densities in Coragen treated and untreated brinjal (eggplant) plots. Ten days following application at 20–60 g a.i. ha\(^{-1}\), counts revealed an average of 2.18 spiders per plant in treated plots and 2.40 spiders per plant in the untreated control. Similarly, Chatterjee and Shanowly (2011) found no significant difference in spider densities between flubendiamide treated and untreated plots of tomato and brinjal. Seven days following application at 60 g a.i. ha\(^{-1}\), counts revealed 9.33 spiders/10 plants in treated and 10.67 spiders/10 plants in untreated brinjal plots, and 6.67 spiders/10 plants in both treated and untreated tomato plots. Studies in Kentucky found no change in spider numbers between chlorantraniliprole treated (230 g a.i. ha\(^{-1}\)) and untreated plots of turfgrass (Larson et al., 2012). The spiders captured in this study predominantly belonged to the families Linyphiidae (sheet web and dwarf spiders) and Lycosidae (wolf spiders). These studies concluded that topical applications of chlorantraniliprole and flubendiamide were compatible for use in IPM and presented little risk to spiders and other beneficial arthropods.

The apparent discrepancies between field studies and lab tests may be attributable to differences in environmental conditions, habitat complexity, and spider species. Niche preferences of spiders may reduce their exposure to pesticides in the field (Pekár, 2012; Yang et al., 2013). For example, ground dwelling spiders (e.g., wolf spiders) may be at less risk of exposure from top-down spraying because of complex foliar coverage acting as a protective barrier. Web-building spiders may be at reduced risk of exposure from topically applied insecticides because webs can effectively buffer and accumulate droplets (Bristowe, 1958; Uetz, 1991; Pekár, 1998). As a foliar dwelling species, *E. ovata* would most likely be at risk of exposure to Coragen residues or by direct topical
contact during field spraying. The dose-response experiment in this study exposed *E. ovata* to both direct topical contact and residues of Coragen. Spiders remained in their initial test dishes following spraying. Studies have found some spiders may avoid surfaces freshly treated with pesticides (Pekár & Haddad, 2005; Pekár & Beneš, 2008; Evans *et al*., 2010); however, *E. ovata* did not display any surface avoidance or web building behaviour following spraying. Oral ingestion may be an additional important route of exposure as tarsal grooming was commonly observed following spraying (pers. observation). Persistence of Coragen in the field depends on temperature, soil alkalinity, and light levels, all of which can impact its degradation and solubility. By-products and derivatives of Coragen are nontoxic, have moderate solubility in water, and no volatility—effectively reducing exposure to non-target arthropods in the field (DuPont Technical Bulletin, 2008). Translaminar movement of Coragen in the field may also reduce contact and exposure from residues, helping to protect crop plants against key pests while minimizing impacts on beneficial arthropods (DuPont Technical Bulletin, 2008). Studies by Vijayasree *et al.* (2015) in India using liquid chromatograph-mass spectrometry (LC-MS/MS) found rates of Coragen applied at 30 and 60 g a.i. ha\(^{-1}\) resulted in surface deposition rates of 0.72 and 1.48 mg kg\(^{-1}\) on brinjal (Solanaceae) and 0.48 and 0.91 mg kg\(^{-1}\) on okra (Malvaceae: Malvaceae). Similar studies by Sharma *et al.* (2014) in India using LC-MS/MS found rates of chlorantraniliprole (Ferterra 0.4G) at 100 and 200 g a.i. ha\(^{-1}\) resulted in soil deposition rates of 0.88 and 1.59 mg kg\(^{-1}\) in sugarcane. Residues dissipated below levels of detection (0.01 mg kg\(^{-1}\)) after 10 days (Vijayasree *et al*., 2015) and 56 days (Sharma *et al*., 2014). In the previously mentioned field studies involving the effects of diamide insecticides on spiders (Chatterjee & Shanowly, 2011; Rajavel *et al*., 2011), evaluation of spider numbers typically occurred several days after application. This would provide ample time for the diamide compounds to dissipate or breakdown (DuPont Technical Bulletin, 2008), after which
unexposed spiders may have migrated back into the field, masking any immediate negative effects. Future studies need to investigate under which circumstances Coragen is most effective at controlling pests while minimizing harm to spiders and other beneficials. Species composition, crop type, abiotic conditions, and pesticide drift all need to be considered (Gennari et al., 1985; Khay et al., 2008).

2.4.2 Sublethal effects of pesticides on spiders

At sublethal concentrations, 0.028 mg mL\(^{-1}\) and 0.056 mg mL\(^{-1}\), Coragen was found to have little effect on predation by adult female *E. ovata*. The number of fruit flies killed by treated and untreated spiders was not statistically different in either of the trials after 4 or 24 h. Although sublethal effects of pesticides on *E. ovata* have not been previously studied, studies have looked at sublethal effects of pesticides in other spiders (See Pekár, 2013). Sublethal effects are characterized as either behavioural and/or physiological changes that result from exposure to survivable levels of pesticides (Desneux et al., 2007; Pekár, 2012). Studies by Samu and Vollrath (1992) found the pyrethroid—cypermethrin, supressed web-building frequency in the cross spider, *Araneus diadematus* (Araneae: Araneidae), and negatively impacted web size and structural accuracy. Pyrethroids, fenvalerate and lambda-cyhalothrin, were also found to compromise locomotion and delay web-building for several days in *Erigone atra* (Araneae: Linyphiidae) and *Oedothorax apicatus* (Araneae: Linyphiidae) (Dinter & Poehling, 1995). Studies by van Erp et al. (2002) found chlorpyrifos, an organophosphorus compound, inhibited cholinesterase activity by as much as 87% in the wolf spider, *Anoteropsis hilaris* (Araneae: Lycosidae). These studies demonstrate the diversity of sublethal effects insecticides can have on spiders. My study is the first to look at the sublethal effects of Coragen on spiders in the laboratory. Therefore, deciding which effect and concentrations
to test was challenging. It is possible that the concentrations I tested were too low to elicit a response with regard to predation, although slight mortality was still observed in spiders at these concentrations indicating Coragen activity (i.e., <47% in trial-1 and <14% in trial-2). Further investigation of the effect of Coragen in relation to predation by *E. ovata* is needed at other sublethal concentrations, especially at concentrations closer to those expected to be encountered in the field (0.5–1.0 mg mL$^{-1}$).

Hormesis is the improved performance of an organism following exposure to low concentrations of pesticides (Pekár, 2012). Such an effect has been observed in *Hylyphantes graminicola* (Araneae: Linyphiidae), *Pardosa amentata* (Araneae: Lycosidae), and *P. pseudoannulata* treated with low concentrations of organophosphates (Toft & Jensen, 1998; Wang *et al*., 2006b; Deng *et al*., 2007). The cause of this improvement in predatory efficiency has not been elucidated and requires further analysis. Although the number of fruit flies killed by *E. ovata* did not differ significantly between treatments, Coragen treated spiders consistently killed more fruit flies on average than untreated spiders. It is possible that low concentrations of Coragen may have resulted in a slight hormetic effect that improved hunting and searching efficiency of prey in adult female *E. ovata*. The mechanism and threshold for this effect remains to be resolved. However, increased activity and movement upon being introduced into the arena may have been a contributing factor to improved hunting success. It is possible that Coragen causes other important sublethal effects in *E. ovata* that were not assessed. Side effects at sublethal concentrations may vary as a result of formulation potency and test species (Han *et al*., 2012). Studies looking at the sublethal effects of chlorantraniliprole in *Plutella xylostella* (Lepidoptera: Plutellidae) (Han *et al*., 2012) and *Spodoptera exigua* (Lepidoptera: Noctuidae) (Xu *et al*., 2010) found significantly reduced pupation rates, pupal
and larval weights, and adult emergence. Further studies should be undertaken to investigate the
effect of Coragen on reproduction, defence, dispersal, development, and mating in *E. ovata*.

2.4.3 Synergistic effects of PBO

In experiment-3, the insecticide synergist PBO was used to investigate the potential metabolic
pathways utilized in the detoxification of Coragen by adult female *E. ovata*. It was found that PBO
acted synergistically with Coragen and resulted in 100% mortality of spiders in treatments where
these compounds were applied in combination (Figure 2.3). The metabolism of Coragen in adult
female *E. ovata* is likely the result of oxidative and/or hydrolytic metabolism. Numerous studies
have reported on the efficacy of PBO in inhibiting critical oxidative and hydrolytic enzymes involved
in detoxification of many insecticides. The inhibitory effect of PBO on oxidative detoxification
enzymes (e.g. mixed-function oxidases (MFOs) like cytochrome P450) has been well characterized in
insects and spider mites by Hodgson and Philpot (1974) as well as by Casida (1970). Studies have
also shown that PBO can inhibit esterases crucial in hydrolytic detoxification in a diversity of insect
species (Young *et al*., 2006; Li *et al*., 2007a; Pereira *et al*., 2014). My study is the first to
demonstrate that metabolic detoxification of this type occurs in spiders and is capable of reducing
susceptibility to diamide insecticides. These findings are consistent with studies investigating
diamide resistance in *Choristoneura rosaceana* (Lepidoptera: Tortricidae) and *Spodoptera exigua*
(Lepidoptera: Noctuidae) which suggested the possibility of enhanced enzymatic detoxification as a
mode of resistance (Lai *et al*., 2011; Sial *et al*., 2011). Studies by Sial *et al*. (2011) found significantly
increased esterase activity in chlorantraniliprole-selected *C. rosaceana* colonies. Similarly, in
laboratory tests, Lai *et al*. (2011) found MFO and esterase activity increased 3-fold and 3.7-fold,
respectively, in chlorantraniliprole-resistant *S. exigua*. Resistance in *S. exigua* to pyrethroids,
organophosphates, and spinosad have all been shown to be the result of enhanced levels of detoxification enzymes (MFOs and esterases) (Delorme et al., 1988; Garza-Urbina & Teran-Vargas, 1998; Wang et al., 2006a). These reports in the literature and my research suggest that enzymatic metabolism of Coragen in arthropods is likely occurring through oxidative and/or hydrolytic detoxification pathways involving MFOs and esterases. Whether or not these pathways are more broadly responsible for resistance to Coragen and other diamides in target pest species remains to be confirmed. Cross-resistance may also be a concern for the long term efficacy of Coragen as pests with enhanced enzymatic metabolism in response to current pesticides may rapidly develop resistance towards Coragen if the same metabolic pathways are involved. Further studies with more specific enzyme inhibitors should be conducted to determine which pathway, oxidative or hydrolytic, is primarily involved in the metabolism of Coragen in *E. ovata* and other spider species.

2.4.4 Conclusion

In conclusion, adult female *E. ovata* were found to be susceptible to topical applications of Coragen at rates at and below those recommended for pest control in field crops by DuPont. There were no apparent sublethal effects of Coragen on the ability of adult female *E. ovata* to kill fruit fly prey in arena tests. Susceptibility of *E. ovata* to Coragen was greatly enhanced when applied in combination with 1% PBO. These findings demonstrate that *E. ovata* are susceptible to Coragen at the ryanodine target site and that detoxification of Coragen is likely occurring through efficient and effective enzymatic metabolism by MFOs and/or esterases. This research serves to broaden our understanding of spider toxicology and to illuminate the effects of a newly introduced class of insecticides on a representative field spider species. Additionally, this research reveals a potential pathway for Coragen resistance through enhanced oxidative and/or hydrolytic enzymatic activity.
This knowledge may serve to better address and direct the future use of Coragen in IPM. Further testing of Coragen on other spider species, genders, and life stages could lend greater insight into the implications of this product on spider communities and agroecosystems as a whole.
Chapter 3

THE EFFECTS OF CORAGEN ON Tibellus SPP. (ARANEAE: PHILODROMIDAE)

3.1 Introduction

In this study, the susceptibility of a common field spider genus, Tibellus (Simon, 1875), to the newly introduced diamide insecticide, Coragen was determined. Pesticide resistance and non-target organism impacts are major concerns for pesticide manufacturers. In order to register and move new compounds into the market, several years of extensive testing are required. Once released, if not managed and rotated properly, these products may become obsolete and discontinued in a shorter amount of time than it took to get them to market. The diamides are one of the most recently introduced groups of insecticides (IRAC Group 28) consisting of two chemical classes: the phthalic diamides (flubendiamide) and the anthranilic diamides (chlorantraniliprole and cyantraniliprole) (Tohnishi et al., 2005; Lahm et al., 2007a; Selby et al., 2013). Diamides are considered ideal for rotation in IPM as they have demonstrated low risk to the environment as well as reduced risk for many non-target organisms (Dinter et al., 2008; DuPont Technical Bulletin, 2008). However, little research has looked at the effects of diamides on spiders, an important group of natural enemies in agroecosystems (Nyffeler & Benz, 1987; Greenstone, 1999; Nyffeler & Sunderland, 2003). Growing popularity and registration of diamides on a global scale puts them at risk of overuse, leading to increased resistance development, and possible harm to non-target organisms (Troczka et al., 2012; Teixeira & Andaloro, 2013). By thoroughly researching these newly introduced compounds and understanding their impacts on non-target arthropods, including beneficial arthropods such as spiders, we can prolong their effective use and minimize potential harm to agroecosystems.
Spiders are key generalist predators that have demonstrated importance in regulating and influencing pest populations in agroecosystems (Nyffeler & Benz, 1987; Sterling et al., 1992; Zhang, 1992; Nyffeler & Sunderland, 2003; Ludy & Lang, 2004). Various broad spectrum insecticides have been shown to negatively impact the ability of spiders to suppress pests through direct lethal and sublethal effects (Pekár, 2012, 2013). Further studies need to be conducted on non-target predatory arthropods such as spiders to broaden our understanding of these newly introduced insecticides and the impacts they might have on crop systems.

*Tibellus* is a genus of spiders belonging to the family Philodromidae, the slender or running crab spiders. They are widespread throughout the world occurring in Europe, Asia, North Africa, and North America (Dondale & Redner, 1978). These spiders are cursorial hunters and actively forage for prey in tall grasses and herbaceous vegetation (Bristowe, 1941). Their preferred habitats are meadows, fields, and roadsides and they are characteristic fauna in the margins of agroecosystems (Dondale, 1961; Samu & Szinetár 2002; Samu et al. 2013). The presence of *Tibellus* in crop systems has been reported by Samu et al. (2001) in cereal fields in Hungary and by Liu et al. (2013) in cabbage fields in Texas. Samu et al. (2013) reported densities of *Tibellus* reaching upwards of 100 spiders $m^{-2}$ in some field margins in northern Hungary. The presence of *Tibellus* on the surface of foliage within crops and crop margins puts them at direct risk of exposure from residues, aerosol applications and pesticide drift (Nentwig et al., 2013). The global distribution and occurrence of *Tibellus* in field crops and margins make them particularly relevant for pesticide toxicity studies. However, no toxicology testing has been previously conducted on this genus.
In this study, the susceptibility of *Tibellus* spp. to the newly introduced diamide insecticide, Coragen was assessed. Topical dose-dependent lethal effects at concentrations corresponding to recommended field application rates were evaluated (experiment-1). Sublethal effects were investigated by evaluating feeding activity, quantified as the rate of killing *Drosophila* spp. over 24 h (experiment-2). The synergistic effect of piperonyl butoxide (PBO) on Coragen was also investigated.

Figure 3.1. An immature female slender crab spider (*Tibellus oblongus*). Body length: 6–9 mm. Photo: Dr. Jonathan Schmidt.
to determine potential detoxification pathways responsible for Coragen metabolism in *Tibellus* spp. (experiment-3).

### 3.2 Materials and Methods

#### 3.2.1 Spider collecting and rearing

*Tibellus* spp. (Araneae: Philodromidae) were collected from couch grass, *Elymus repens* (L.) Gould (Poales: Poaceae), at sites in Elora (43.65588N, 80.41452W) and Puslinch (43.460976N, 80.273899W), Ontario, Canada from July to October of 2013 and 2014. Late instar and adult female spiders dominated the field population sampled in both years. Spiders were captured by sweep netting along 150–200 m transects. Spiders were placed into either 1.5 mL microcentrifuge tubes or 15 mL conical centrifuge tubes (Fisher Scientific, Ottawa, Canada). Spiders were later transferred to 100 × 25 mm plastic Petri dishes (Fisher Scientific, Ottawa, Canada) where they were individually housed and fed until used in experiments. Each dish contained a piece of paper towel for substrate and a cotton swab to which deionized water was applied weekly. The spiders were fed two fruit flies (*Drosophila* spp. (Diptera: Drosophilidae)) each week (See Appendix 1: Fruit Fly Rearing). Feeding usually coincided with watering. All spiders used in toxicity tests were starved for one week before experiments to standardize satiation. During the 2013 field season, spiders were kept in a growth room with controlled temperature and humidity (17 ± 1°C, 60 ± 8% RH). The cool temperature of the growth room was intended to slow the metabolism and energy demands of the spiders, and to prolong their longevity (Foelix, 2010). High ambient humidity was essential to mitigate dehydration and desiccation. During the 2014 field season, spiders were maintained at ambient lab conditions (21.5 ± 1°C, 50 ±8 % RH). There were no differences in the mortality of spiders in the control treatments during the course of testing between years (*i.e.*, 0% in both years).
Temperature and humidity measurements were taken using a Traceable™ thermometer / clock / humidity monitor (Fisher Scientific, Ottawa, Canada).

3.2.2 Spider selection for testing

Prior to experimentation, spiders were sorted based on gender and life stage. Gender was determined by examining the pedipalps of the spiders with the naked eye. Further, females have smooth cylindrical distal tarsi, whereas males have clubbed or bulbous distal tarsi (Dondale & Redner, 1978; Foelix, 2010; See Appendix 4: Sexing Spiders by Pedipalps). Females of the same maturity and species were identified using “The Insects and Arachnids of Canada Part 5: The Crab Spiders of Canada and Alaska (Araneae: Philodromidae and Thomisidae)” key (Dondale & Redner, 1978). Two species dominated the collection sites, *Tibellus oblongus* (Walckenaer, 1802) and *Tibellus maritimus* (Menge, 1875). Both species were used in toxicity tests. Only adult female spiders were selected for toxicity tests. Female spiders are generally larger than males or juveniles and have been reported to have a higher tolerance to pesticides (Dinter & Poehling, 1995; Deng et al., 2006). Males are relatively short-lived and would have higher background mortality in toxicity tests than adult females (Pekár, 2012). Spiders were randomly assigned to treatment groups in all experiments using a random number generator. This consisted of giving each spider a unique number and populating each treatment group based on the random number generated.

3.2.3 Chemicals tested

Spray formulations were prepared from a stock solution of 200 g L$^{-1}$ Coragen obtained from DuPont Canada (Mississauga, Ontario, Canada). Dilutions were prepared with deionized water to obtain desired concentrations. The stock solution contained a guaranteed active ingredient concentration of 18.4% chlorantraniliprole (CAS # = 500008-45-7) (3-Bromo-N-[4-chloro-2methyl-6-
[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide). The methylenedioxyphenyl synergist piperonyl butoxide (PBO) (CAS # = 51-03-6) (5-[2(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole) was obtained from Acros Organics (New Jersey, USA). Formulations were prepared from a stock solution of 90% PBO through a two-phase dilution series. One mL of 90% PBO was initially diluted with 95% ethanol to a total volume of 10 mL. This was followed by a second dilution with deionized water to a total volume of 90 mL to obtain a 1% PBO solution. Formulations were prepared fresh before each test.

3.2.4 Spray apparatus and application

Coragen, PBO, and water were applied topically to spiders as aerosols using a miniature spray tower. The spray tower consisted of a metal tube with an Iwata® Eclipse HP-BCS airbrush (Wyndham Art Supplies, Guelph, Canada) centred at the top of it (Gradish et al., 2009a, 2009b). An air compressor delivering 15 PSI powered the apparatus. The spray pattern was tested and calibrated by counting droplets on water-sensitive paper (Syngenta, Basel, Switzerland) for a 1 mL volume of deionized water. The sprayer delivered a conical spray pattern that evenly stained the test paper with a distribution of about 30–50 droplets cm\(^{-2}\). Further testing of the spray tower was conducted to determine the actual deposition rate for a 1 mL load of solution (See Appendix 2: Spray Tower Calibration). Based on these measurements, it was determined that 0.0214 ± 0.0009 mL of solution were deposited over the target area (28.274 cm\(^2\)) during each application.

Spiders were placed individually into 60 × 15 mm Pyrex® Petri dishes (Fisher Scientific, Ottawa, Canada) prior to being sprayed. Each dish was lined with a 4.7 cm diameter Whatman #5 filter paper (Fisher Scientific, Ottawa, Canada) as a substrate. Prior to spraying, spiders were placed
in a freezer at -18 ± 1°C for 7 min to immobilize them during spraying. Freezing and spraying typically occurred in groups of 5 to ensure that spiders did not become active prior to treatment. For each treatment the spray tower was loaded with 1 mL of either water, Coragen, or PBO using an Oxford® BenchMate® II single-channel, piston-driven, air-displacement pipette with disposable tips (Nichiryo, Maryland Heights, USA). The spray tower was cleansed with acetone and deionized water between treatments to eliminate the potential for cross-contamination. All tests were isovolumetric and used 1 mL of solution to ensure similar saturation of the test dish surface. A volume of 1 mL was selected as higher volumes caused pooling on the filter paper.

3.2.5 Experiment 1: Dose-response testing

Coragen was tested at concentrations ranging from 0.113–1.125 mg mL\(^{-1}\) which corresponded to corrected application rates of 8.51–85.15 g ha\(^{-1}\) (Table 3.1). These ranges included the recommended tank mixture concentrations (0.5–1.0 mg mL\(^{-1}\)) and were similar to the recommended field application rates (50–100 g ha\(^{-1}\)) (DuPont Supplemental Label, 2010). The number of spiders tested at each treatment level was 20 in 2013 and 15 in 2014. Deionized water was used as a negative control in each test series. The mortality of spiders was assessed by checking for responsiveness at 1, 24, and 48 h after spraying. A small, metal probe was used to poke the dorsal side of the abdomen to elicit a response. Knockdown (paralysis) was expected to occur within minutes to a few hours after spraying (DuPont Technical Bulletin, 2008; Bassi et al., 2009; Hamaguchi and Hirooka, 2012). Following the 48 h check, unresponsive spiders were considered to be dead. Spiders were preserved in 95% ethanol for future reference or measurements.
Table 3.1. Tested concentrations and corresponding application rates of Coragen (active ingredient = chlorantraniliprole; DuPont).

<table>
<thead>
<tr>
<th>Concentrations tested (mg/mL)</th>
<th>Corrected rates (μg/cm²)a</th>
<th>Equivalent field rates (g/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.113</td>
<td>0.085</td>
<td>8.51</td>
</tr>
<tr>
<td>0.281</td>
<td>0.213</td>
<td>21.29</td>
</tr>
<tr>
<td>0.563b</td>
<td>0.426</td>
<td>42.57</td>
</tr>
<tr>
<td>1.125b</td>
<td>0.852</td>
<td>85.15</td>
</tr>
</tbody>
</table>

aAccounting for area of test dish (A = 28.274 cm²) and 2.14% spray tower correction factor (Appendix 3).

bLast two rows represent concentrations and tank mix rates similar to those recommended by DuPont.

3.2.6 Experiment 2: Testing sublethal effects of Coragen on predation

Coragen at a concentration of 0.056 mg mL⁻¹ caused less than 50% mortality in preliminary tests and was selected for testing sublethal effects on feeding behaviour. Spiders were treated according to protocol in experiment-1. Control spiders were treated with deionized water in each test series. Only spiders that were responsive 2 h after spraying were used for feeding trials. Spiders were left for 2 h in the initial test dishes. Spiders were subsequently transferred to larger 100 × 25 mm plastic Petri dishes (Fisher Scientific, Ottawa, Canada) which contained a piece of paper towel for substrate and a cotton swab saturated with deionized water for moisture. The use of different dishes ensured that the fruit flies would not be exposed to Coragen residues. For the feeding tests, each spider was provided with 10 ten fruit flies. Dishes containing only fruit flies were also prepared to monitor the background mortality of fruit flies. Fly mortality was checked at 4 and 24 h after they had been provided to the spiders. An initial check at 4 h was chosen because *Tibellus* spp. feed primarily within the first few minutes to hours after being introduced to prey (pers. observation). The 24 h check was to monitor the status of the treated spiders. The mortality of spiders was assessed as in experiment-1 and only data for responsive spiders was included in the analysis. Spiders were preserved in 95% ethanol following completion of the experiment.
3.2.7 Experiment 3: Testing the synergistic effects of PBO

Four treatments were tested: water, 1% PBO alone, Coragen alone, and 1% PBO and Coragen in combination. Coragen was applied at two different concentrations: 0.056 mg mL\(^{-1}\) in trial-1 and 0.028 mg mL\(^{-1}\) in trial-2. The concentration of PBO tested in both trials was 1% (10 mg mL\(^{-1}\)). This concentration of PBO was selected based on the high end of typical co-application rates with pyrethrin and pyrethroid insecticides. In preliminary tests, no mortality was observed after 48 h with 2% PBO. The combined application of PBO and Coragen occurred sequentially. Spiders were initially divided into two groups and sprayed with either deionized water or 1% PBO. Two hours later, each of those groups were divided again and sprayed with deionized water and Coragen. This sequential two stage spraying method allowed me to test every permutation of water, PBO, and Coragen (\emph{i.e.}, water-water, water-Coragen, 1% PBO-water, and 1% PBO-Coragen). Fifteen spiders were tested at each treatment level. Assessing the mortality of spiders and the preservation of dead spiders proceeded as in the previous experiments.

3.2.8 Statistical analysis

For experiment-1, treatment concentrations were converted using a log transformation in order to acquire a linear relationship between Probit mortality and concentration. A Probit regression analysis was then conducted to calculate the \(\text{LC}_{50}\) value with corresponding 95% confidence intervals. A Chi-square test was used to determine the goodness-of-fit of the observed values to the predicted values in the Probit regression. In experiment-2, a nonparametric Mann Whitney U test was used to compare a continuous outcome (mortality) with two independent samples (Coragen treated and untreated spiders). A nonparametric Mann Whitney U test was selected instead of a standard \(t\)-test for independent samples because sample sizes were smaller
than 30 and normality could not be assumed. The Mann Whitney U test assumes homogeneity of variance, and this was verified for each treatment using a nonparametric Levene’s test (p>0.05) (Nordstokke & Zumbo, 2010; Nordstokke et al., 2011). IBM SPSS Ver. 22.0 (IBM Corp., Armonk, NY) was used to perform all statistical computations. A Type I error of 0.05 was used for all statistical tests.

3.3 Results

3.3.1 General observations of the effects of Coragen

*Tibellus* spp. were observed over a period of 48 h after spraying with Coragen or water. Both Coragen- and water-treated spiders exhibited tarsal grooming which lasted approximately 30–50 min, following exposure. Coragen treated spiders consistently moved about in their test dishes for a few hours following spraying, often settling upside down on the lid. Following this, *Tibellus* displayed little or no subsequent movement unless disturbed with a metal probe. After 24 h, those treated with higher concentrations of Coragen did not react when disturbed and instead maintained a rigid posture with all legs outstretched.

3.3.2 Experiment 1: Dose-response testing

Over the range of concentrations tested in 2013 and 2014, mortality in adult female *Tibellus* spp. increased with increasing concentrations of Coragen (Table 3.3, 3.4). In preliminary range finding tests, mortality was 30% at 0.113 mg mL\(^{-1}\) and 80% at 1.125 mg mL\(^{-1}\) after 24 h. Control mortality was 0% after 48 h in all trials. In definitive tests, no mortality was observed 1 h after spraying. Mortality usually increased between 1 and 24 h, especially at higher concentrations of Coragen. At all concentrations, mortality reached a peak after 24 h except in 2014 at 0.113 mg mL\(^{-1}\).
where mortality was found to increase between 24 and 48 h. This suggests that it takes several hours for Coragen to take effect in adult female *Tibellus*, even at higher concentrations. At 48 h, Coragen tested at 0.563 mg mL\(^{-1}\) resulted in 50% mortality in 2013 and 33% mortality in 2014, and at 1.125 mg mL\(^{-1}\) the resulting mortality was 85% in 2013 and 73% in 2014 (Table 3.3, 3.4).

According to standards set by the International Organization of Biological Control (IOBC), these findings indicate that Coragen is slightly harmful (25–50% mortality) to harmful (>75% mortality) to adult female *Tibellus* spp. at rates comparable to those recommended for field application (Hassan, 1985). The harmfulness of Coragen was further corroborated by a roughly calculated LD\(_{50}\) based on surface area estimates and measured wet weights of adult female *Tibellus* (See Appendix 3: Theoretical LD\(_{50}\) (mg insecticide per kg of body weight) for *Tibellus*). The estimated LD\(_{50}\) was 29 mg kg\(^{-1}\), a dosage considered to be extremely hazardous for humans and other mammals according to World Health Organization standards and toxicity classifications (WHO, 2010). The extrapolated field rate corresponding to 0.563 mg mL\(^{-1}\) is 42.57 g ha\(^{-1}\) and for 1.125 mg mL\(^{-1}\) it is 85.15 g ha\(^{-1}\) (Table 2.1), rates comparable to those recommended by DuPont, 50–100 g ha\(^{-1}\) (DuPont Supplemental Label, 2010). The results demonstrate that the RyRs of *Tibellus* spp. are susceptible to Coragen at concentrations similar to those recommended for use against many target insect pest species (DuPont Technical Bulletin, 2008; DuPont Supplemental Label, 2010).

The log-probit LC\(_{50}\) value was 0.583 mg mL\(^{-1}\) with a 95% confidence interval of 0.470 to 0.750 mg mL\(^{-1}\). This included mortality data from both 2013 and 2014. The log-probit model displayed adequate fit for the relationship between dosage concentrations and observed and fitted probabilities with a Pearson Goodness-of-Fit p-value of 0.071 (Table 3.2).
Table 3.2. Probit table for the effects of Coragen (active ingredient = chlorantraniliprole) 24-h after exposure to adult female *Tibellus* spp. in 2013 and 2014.

<table>
<thead>
<tr>
<th>Conc. (mg/mL)</th>
<th>Log of Conc.</th>
<th>Number of spiders tested</th>
<th>Total observed mortality</th>
<th>Total expected mortality</th>
<th>Residual Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0.000</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.113</td>
<td>-0.949</td>
<td>20</td>
<td>1</td>
<td>0.588</td>
<td>0.412</td>
</tr>
<tr>
<td>0.113</td>
<td>-0.949</td>
<td>15</td>
<td>0</td>
<td>0.441</td>
<td>-0.441</td>
</tr>
<tr>
<td>0.281</td>
<td>-0.551</td>
<td>20</td>
<td>8</td>
<td>4.029</td>
<td>3.971</td>
</tr>
<tr>
<td>0.281</td>
<td>-0.551</td>
<td>15</td>
<td>0</td>
<td>3.021</td>
<td>-3.021</td>
</tr>
<tr>
<td>0.563</td>
<td>-0.250</td>
<td>20</td>
<td>10</td>
<td>9.677</td>
<td>0.323</td>
</tr>
<tr>
<td>0.563</td>
<td>-0.250</td>
<td>15</td>
<td>5</td>
<td>7.258</td>
<td>-2.258</td>
</tr>
<tr>
<td>1.125</td>
<td>0.051</td>
<td>20</td>
<td>17</td>
<td>15.502</td>
<td>1.498</td>
</tr>
<tr>
<td>1.125</td>
<td>0.051</td>
<td>15</td>
<td>11</td>
<td>11.627</td>
<td>-0.627</td>
</tr>
</tbody>
</table>

(Total number of spiders tested = 175)

Table 3.3. Summary of dose-response data for adult female *Tibellus* spp. exposed to Coragen (active ingredient = chlorantraniliprole) applications in 2013.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>No. of Spiders Tested</th>
<th>% Mortality (1-h after spraying)</th>
<th>% Mortality (24-h after spraying)</th>
<th>% Mortality (48-h after spraying)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.113</td>
<td>20</td>
<td>0.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>0.281</td>
<td>20</td>
<td>0.0</td>
<td>40.0</td>
<td>40.0</td>
</tr>
<tr>
<td>0.563</td>
<td>20</td>
<td>0.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>1.125</td>
<td>20</td>
<td>0.0</td>
<td>85.0</td>
<td>85.0</td>
</tr>
</tbody>
</table>

(Total number of spiders tested = 100)

Table 3.4. Summary of dose-response data for adult female *Tibellus* spp. exposed to Coragen (active ingredient = chlorantraniliprole) applications in 2014.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>No. of Spiders Tested</th>
<th>% Mortality (1-h after spraying)</th>
<th>% Mortality (24-h after spraying)</th>
<th>% Mortality (48-h after spraying)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>15</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.113</td>
<td>15</td>
<td>0.0</td>
<td>0.0</td>
<td>6.7</td>
</tr>
<tr>
<td>0.281</td>
<td>15</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.563</td>
<td>15</td>
<td>0.0</td>
<td>33.3</td>
<td>33.3</td>
</tr>
<tr>
<td>1.125</td>
<td>15</td>
<td>0.0</td>
<td>73.3</td>
<td>73.3</td>
</tr>
</tbody>
</table>

(Total number of spiders tested = 75)
3.3.3 Experiment 2: Testing sublethal effects of Coragen on predation

Very few fruit flies (<1%) were dead after 24 h in dishes that did not contain spiders, indicating that most or all of the observed fruit fly mortality can be attributed to predation by spiders in the treatments. No fruit fly deaths were observed at 4 h in dishes without spiders. At 24 h, a single fruit fly was found dead in the dishes without spiders (i.e., 0.1 fruit flies dead per dish). Background fruit fly mortality was corrected with Abbott’s formula.

A sublethal application of Coragen at 0.056 mg mL\(^{-1}\) had no negative effect on *Tibellus* spp. predation on *Drosophila* spp. (Figure 3.2). Coragen treated spiders were found to kill a greater number of fruit flies on average than untreated spiders. This was evident after 4 h of hunting time. After 4 h of hunting time, Coragen treated spiders killed 6.5 ± 0.4 fruit flies on average, whereas untreated spiders killed 4.3 ± 0.5 fruit flies on average. This trend continued after 24 h of hunting time, Coragen treated spiders killed 10.6 ± 0.6 fruit flies on average, whereas untreated spiders killed 8.0 ± 0.7 fruit flies on average. At 4 h, a Mann-Whitney U test indicated the mean rank for untreated spider predation (14.78) was significantly lower than the mean rank for treated spider predation (25.50) (\(U=85.5, n_1=20, n_2=19, Z=-2.963, p=.002\)). At 24 h, the mean rank for untreated spiders was not statistically significantly different from treated spiders based on a Mann-Whitney U test (\(p>0.05\)).

The mortality of spiders was comparable to those observed in experiment-1 at similar exposure levels. In this experiment, the mortality of spiders was 5% after treatment with Coragen at a concentration of 0.056 mg mL\(^{-1}\), comparable to the 5% mortality observed at 0.113 mg mL\(^{-1}\) in experiment-1 for 2013. All control spiders survived treatment with deionized water.
3.3.4 Experiment 3: Testing the synergistic effects of PBO

In two trials conducted during 2014, mortality 48 h after treatment was highest for spiders treated with a combination of Coragen (0.056 or 0.028 mg mL⁻¹) and 1% PBO compared to those treated with either 1% PBO (<15%) or Coragen alone (0%) (Figure 3.3). All control spiders (water-treated group), with the exception of a single individual in trial-1, survived treatment with deionized water. Treatment with 1% PBO alone caused less than 15% mortality in trial-1 and no mortality in trial-2. Mortality caused by Coragen alone was comparable to that observed for similar concentrations used in experiment-1 (Table 3.3, 3.4).
3.4 Discussion & Conclusion

Adult female *Tibellus* spp. were susceptible to topical applications of Coragen at applied rates comparable to the recommended tank concentrations (0.5–1.0 mg mL\(^{-1}\)) and field application rates (50–100 g ha\(^{-1}\)) provided by DuPont (DuPont Supplemental Label, 2010; DuPont Technical Bulletin, 2008) (Table 3.1). Mortality ranged from 33–85\% at concentrations of 0.563 and 1.125 mg mL\(^{-1}\) in 2013 and 2014 (Table 3.3, 3.4), classifying Coragen as slightly harmful to harmful according to IOBC standards (Hassan, 1985). As in the case of *E. ovata*, the effect of Coragen was evident from the the ataxia and paralysis observed after treatment, which are the same effects reported for other
arthropod species (Cordova et al., 2006; DuPont Technical Bulletin, 2008; Bassi et al., 2009; Hamaguchi and Hirooka, 2012). These symptoms typically occurred within the first 24 h after Coragen exposure and were consistent with those observed for susceptible insects (Lahm et al., 2005; Cordova et al., 2006; DuPont Technical Bulletin, 2008; Satelle et al., 2008). These results indicate that Coragen acts with the same mode of action and at the same molecular target site in *Tibellus* spp. as it does in insects. Differences between the RyRs of the target pests and *Tibellus* appear to be insufficient to result in selectivity. This may suggest partial or complete conservation of genes coding for the RyRs in these arthropods. Residues (183–290) coding a portion of the N-terminal region of lepidopteran RyRs were found to be structurally required for flubendiamide-induced activation and that this region shared 94–99% identity with other insects (Kato et al., 2009; Cui et al., 2013). This extent of genetic conservation between pests and non-target organisms remains to be investigated in regard to chlorantraniliprole activation of RyRs in other classes of arthropods, including spiders (Arachnida). However, the results observed for *Tibellus* and *E. ovata* (Chapter 2) indicate that the main structural features of RyRs in arthropods may be highly conserved and differentiated from those of vertebrates.

The estimated LC$_{50}$ for adult female *Tibellus* was approximately 583 mg L$^{-1}$ with a 95% confidence interval of 470 to 750 mg L$^{-1}$. This is considerably greater than the estimated LC$_{50}$ determined for *E. ovata* in Chapter 2 (210 mg L$^{-1}$ with a 95% confidence interval of 161 to 279 mg L$^{-1}$). Differences in susceptibility between these two families of spiders may be due to behavioural or physiological differences. Both types of spiders were tested under identical laboratory conditions, eliminating differences in niche preference as a potential explanation for differing susceptibilities that may have otherwise been difficult to control in field tests (Rodrigues et al., 2013). Immediately following spraying, *Tibellus* spp. were considerably more active than *E.*
and were found to relocate to the lids of their test dishes. This behaviour may be indicative of surface avoidance, when an organism avoids a chemically treated surface because residues act as a deterrent or repellent (Pekár, 2012). This behaviour may explain the higher observed tolerance to Coragen in *Tibellus* than *E. ovata* (Pekár & Haddad, 2005; Pekár & Beneš, 2008; Evans et al., 2010). Alternatively, these two families of spiders may differ from one another with respect to penetration or detoxification. Differences in RyRs susceptibility may also be involved, but is less likely a major factor. The factors involved could be elucidated through protein crystallography/microscopy of RyRs and analysis of intrinsic detoxification enzyme levels between the two spiders. The results obtained here are consistent with the differing susceptibilities observed for two spider families treated with flubendiamide in laboratory tests by Lahm et al. (2007b). Lahm et al. (2007b) found EC$_{30}$ values of $>100$ mg a.i. L$^{-1}$ for *Pardosa pseudoannulata* (Araneae: Lycosidae) and $>200$ mg a.i. L$^{-1}$ for *Misumenops tricuspidatus* (Araneae: Thomisidae). These findings suggest that spiders are vulnerable to diamide insecticides at the ryanodine target site and that susceptibility may differ depending on the specific compound and spider species. There have been few studies that have looked at the impact of diamides on spiders in the field; among the few that have, no negative effects on spider density have been reported (Chatterjee & Shanowly, 2011; Rajavel et al., 2011; Larson et al., 2012). However, these studies failed to investigate the direct effects of diamides on spiders immediately following exposure, often reporting numbers several days after application. Delayed evaluation of spider numbers would provide ample time for the diamide compounds to dissipate or breakdown (DuPont Technical Bulletin, 2008), after which unexposed spiders may have migrated back into the field, masking any immediate negative effects. In the field, *Tibellus* would most likely be at risk of exposure to Coragen residues or by direct topical contact from spraying. However, they may be at less risk of exposure from top-down spraying because of complex foliar
coverage acting as a protective barrier. More thorough studies need to investigate the effects of these compounds over a variety of time intervals and on different types of spiders.

In this study, a sublethal concentrations of Coragen at 0.056 mg mL$^{-1}$ was not found to negatively affect feeding behaviour of adult female Tibellus. At 4 h, Coragen treated spiders killed significantly more fruit flies on average than untreated spiders. During the subsequent 20 h of hunting time, treated spiders and untreated spiders killed similar numbers of fruit flies. Although sublethal effects of pesticides on Tibellus have not been previously studied, there have been studies looking at sublethal effects of pesticides in other spiders (See Pekár, 2013). Sublethal effects are characterized as either behavioural and/or physiological changes as a result of exposure to survivable levels of pesticides (Desneux et al., 2007; Pekár, 2012). In many cases, sublethal concentrations of insecticides have been found to have deleterious effects on spiders. Samu and Vollrath (1992) found the pyrethroid insecticide, cypermethrin, supressed web-building frequency in cross spiders, Araneus diadematus (Araneae: Araneidae), and negatively impacted web size and structure. Pyrethroids, fenvalerate and lambda-cyhalothrin, also compromised locomotion and delayed web-building for several days in Erigone atra (Araneae: Linyphiidae) and Oedothorax apicatus (Araneae: Linyphiidae) (Dinter & Poehling, 1995). Studies by Van Erp et al. (2002) found the organophosphate, chlorpyrifos, inhibited cholinesterase activity by as much as 87% in the wolf spider Anoterpopsis hilaris (Araneae: Lycosidae). In contrast, the current study found Coragen treated spiders killed significantly more fruit flies than untreated spiders. These findings may indicate a hormetic effect, the improved performance of an organism following exposure to low dosages of pesticides (Pekár, 2012). A similar effect was observed in Hylyphantes graminicola (Araneae: Linyphiidae), Pardosa amentata (Araneae: Lycosidae), and P. pseudoannulata treated with low dosages of organophosphates (Toft & Jensen, 1998; Wang et al., 2006b; Deng et al., 2007).
The basis for this improved predatory efficiency is unknown and requires further analysis. However, it is possible that the increased locomotion (avoidance) or sensitivity following exposure initially increases searching efficiency and hunting success. The mechanism and threshold for this effect remains to be resolved. Similar findings were made in Chapter 2 when observing *E. ovata* after 4 and 24 h of hunting time, suggesting this effect may be consistent over different spider families.

Further studies need to be conducted for Coragen at other sublethal concentrations and on other spider families to determine the consistency of these findings. It is possible that Coragen has other sublethal effects on *Tibellus* that were not assessed. Future studies need to investigate the effect of Coragen on fertility, defence, dispersal, development, and mating success.

In experiment-3, mortality was found to be greatest in spiders treated with a combination of PBO and Coragen. Pretreatment with PBO renders *Tibellus* susceptible to concentrations of Coragen that are otherwise minimally toxic, demonstrating that PBO acts as a synergist for Coragen. PBO is a known inhibitor of MFOs and esterases (Casida, 1970; Hodgson and Philpot, 1974; Young et al., 2006; Li et al., 2007a; Pereira et al., 2014). MFOs and esterases play an important role in the metabolism and detoxification of xenobiotics within most eukaryotic organisms. It is evident that adult female *Tibellus* depend on these pathways in order to metabolize Coragen. This is consistent with the result obtained for *E. ovata* (Chapter 2: Experiment 3). These findings are important to consider with respect to the development of resistance in arthropods generally. Studies by Sial et al. (2011) found significantly increased esterase activity in chlorantraniliprole-selected *C. rosaceana* colonies. Similarly, in laboratory tests, Lai et al. (2011) found MFO and esterase activity increased 3-fold and 3.7-fold, respectively, in chlorantraniliprole-resistant *S. exigua*. Resistance in *S. exigua* to pyrethroids, organophosphates, and spinosad have all been shown to be the result of enhanced levels of detoxification enzymes (MFOs and esterases) (Delorme et al., 1988; Garza-Urbina & Teran-
Vargas, 1998; Wang et al., 2006a). These reports in the literature and my research suggest that enzymatic metabolism of Coragen in arthropods may generally be occurring through oxidative and/or hydrolytic detoxification pathways involving MFOs and esterases.

In conclusion, adult female *Tibellus* spp. were found to be susceptible to topical applications of Coragen at rates at and below those recommended by DuPont for target insect pest control in field crops. At sublethal concentrations, Coragen treated spiders killed more fruit flies on average than untreated spiders in arena tests. A possible hormetic effect could account for this result due to increased activity following spraying (surface avoidance behaviour) or agitation upon being transferred to a new arena. Susceptibility of *Tibellus* to Coragen was greatly enhanced when applied in combination with 1% PBO. These findings demonstrate that *Tibellus* are susceptible to Coragen at the ryanodine target site and that detoxification of Coragen is likely occurring through efficient and effective enzymatic metabolism by mixed-function oxidases and/or esterases. This research serves to broaden our understanding of spider toxicology and to illuminate the effects of a newly introduced class of insecticides on a representative field spider species. Additionally, this research reveals a potential pathway for Coragen resistance through enhanced oxidative and/or hydrolytic enzymatic activity. This knowledge may serve to better address and direct the future use of Coragen in IPM. Further testing of Coragen on other spider species, genders, and life stages could lend greater insight into the implications of this product on spider communities and agroecosystems as a whole.
Chapter 4

GENERAL DISCUSSION AND CONCLUSIONS

4.1 Differences in the susceptibility of spiders to insecticides: Lab vs. field

Spiders are important natural enemies present in a variety of ecosystems (Turnbull, 1973; Wise, 1993). They are one of the most numerically dominant arthropod groups in agroecosystems and have demonstrated the ability to control and influence pest populations (Nyffeler & Benz, 1987; Greenstone, 1999; Nyffeler & Sunderland, 2003). These generalist predators are of great importance for IPM because of their natural ability to control various insect crop pests without being a detriment to the crop plants themselves (Rodrigues et al., 2009). However, the use of broad-spectrum non-selective insecticides has been shown to negatively affect beneficial arthropods, including spiders (Croft, 1990; Desneaux et al., 2007; Pekár, 2012). In many studies, a reduction in the abundance of natural enemies has been associated with increased damage to crops from pest resurgences and secondary outbreaks (Fritz et al., 1990; Ruberson et al., 1998; Tanaka et al., 2000; Yu et al., 2008). Most broad-spectrum insecticides (e.g., organophosphates, carbamates, pyrethroids, and cyclodienes) as well as acaricides have been shown to interfere with the ability of spiders to control pests (Mansour & Nentwig, 1988; Pekár, 2012). Preserving natural enemies is important for IPM. Therefore, it is important to study the impacts of novel insecticides on a diversity of spiders typically present in agroecosystems, to ensure that detrimental long term impacts on these beneficial arthropods are mitigated.

Coragen is a recently introduced ryanoid insecticide that was released in North America by DuPont in 2008. This product has demonstrated low impact on mammals due to its high selectivity for arthropod RyRs (Lahm et al., 2007a). It has also demonstrated a favourable environmental
profile due to its degradation into nontoxic derivatives when exposed to high temperatures, alkaline soil conditions, and ultraviolet light in the field (DuPont Technical Bulletin, 2008). DuPont makes the general claim that Coragen has a negligible impact on key natural enemies (i.e., predators and parasitoids), and pollinators at recommended field rates; however, their list of tested predators fails to include true spiders (Order: Araneae) (DuPont Technical Bulletin, 2008). Independent studies have reported little effect of diamide insecticides on spider densities in the field (Chatterjee & Shanowly, 2011; Rajavel et al., 2011; Larson et al., 2012). The only field study that did find some effect of chlorantraniliprole on spider densities was that by Yang et al. (2014) in maize fields, in which chlorantraniliprole reduced $X. \text{ ephippiatus}$ populations three days after applications at 48.1 mg L$^{-1}$ by as much as 33%. Nonetheless, all of these studies concluded that diamide insecticides were harmless to spiders in the field and thus, would integrate well with IPM strategies. My current study serves to broaden our understanding of the effects of Coragen on spiders through laboratory testing.

I found that two spiders, $Enoplogantha ovata$ and $Tibellus$ spp., were susceptible and negatively affected by topical applications of Coragen at concentrations similar to recommended tank concentrations and field application rates in laboratory tests. Although $Tibellus$ was found to be more tolerant to topical applications of Coragen than $Enoplognatha$, both species were susceptible to Coragen. These findings suggest that Coragen lacks selectivity between intended insect pests and spiders, likely as the result of similarities in the RyR target sites of these arthropod groups. Slight structural differences at the RyR binding site or physiological differences in levels of intrinsic detoxification enzymes may account for the observed variation in susceptibility between $Enoplognatha$ and $Tibellus$ (Tanaka et al., 2000; Markó et al., 2009; Pekár, 2012). Further analyses would need to be conducted to determine the basis for similarities between spiders and target
pests, and for differences in tolerance among spiders. For example, structural differences in the
target site could be investigated using spectroscopic or crystallographic techniques (Aidley &
Stanfield, 1996), whereas levels of detoxification enzymes could be investigated through
biochemical analyses (Brooke et al., 2001).

It is important to assess the effects of pesticides on various types of spiders, since it is
assemblages of spiders rather than individual spider species that generally contribute to the control
of pests within agroecosystems (Riechert, 1999). Not all species of spiders are affected by
insecticides in the same way and effects may differ depending on guild or family (Pekár, 2012). For
example, studies in apple orchards in southern England by Markó et al. (2009) found that sheet and
orb weaving guilds were negatively affected by broad-spectrum insecticides, but spiders in the
space-web guild were not. Similar studies by Tanaka et al. (2000) in field and laboratory tests in
Japan found the organothiophosphate—phenthoate, reduced only cursorial spider numbers,
whereas the pyrethroids—deltamethrin and etofenprox, reduced only sheet-web (Araneae:
Linyphiidae) and orb-web spiders (Araneae: Araneidae). In these studies, it was argued that
ecological (e.g., niche preferences such as foliar versus ground dwelling) or physiological differences
(e.g., intrinsic levels of detoxification enzymes) likely accounted for the varying susceptibilities
observed among different spiders treated with the same insecticides (Tanaka et al., 2000; Markó et
al., 2009; Pekár, 2012). However, some insecticides can adversely affect the entire spider
community. For example, the organophosphate—formothion, and the carbamate—carbaryl,
negatively impacted all members of spider communities in both apple orchards and citrus groves in
Israel (Mansour, 1987b, 1988). By studying the effects of Coragen on multiple species of spiders, a
more thorough understanding of its effects on the spider community could be obtained. It will be
important to determine whether the effects of Coragen are consistent over the entire spider community or if my observations are limited to *Enoplognatha ovata* and *Tibellus* spp.

The conditions and environments in which pesticides are tested can greatly influence their observed effects (Markó *et al.*, 2009). That is, spiders tested in a laboratory may not necessarily display the same susceptibility to a given chemical as those tested in the field. This is frequently due to differences in routes of exposure (Markó *et al.*, 2009; Pekár, 2012). Studies in the field may provide the most realistic circumstances for evaluating exposure to pesticides and their consequences, but these studies sometimes fail to identify and distinguish specific causes for direct mortality and side effects. The dose-response assays conducted in this thesis consisted of direct topical application of Coragen to spiders using a spray tower. However, residual contact may have also been a major route of exposure since spiders remained in their test dishes after spraying. *Tibellus* displayed behaviour consistent with surface avoidance shortly after spraying; they were often found upside down on the lids of their test dishes (pers. observation). This might have reduced contact with residues resulting in a higher observed tolerance to Coragen compared to *Enoplognatha*, which did not display this behaviour. Avoidance of pesticide treated surfaces by spiders has been well studied (Pekár & Haddad, 2005; Pekár & Beneš, 2008; Evans *et al.*, 2010). For example, spiders of the genus *Pardosa* (Araneae: Lycosidae) have been found to avoid surfaces treated with the herbicide—glyphosate (Evans *et al.*, 2010), as well as other insecticides, including permethrin, phosalone (Pekár & Haddad, 2005), deltamethrin, and a mixture of chlorpyrifos and cypermethrin (Pekár & Beneš, 2008). Studies by Pekár and Haddad (2005) further concluded that spiders of the genera *Clubiona* (Araneae: Clubionidae), *Dictyna* (Araneae: Dictynidae), *Philodromus* (Araneae: Philodromidae) and *Xysticus* (Araneae: Thomisidae) also avoided fresh residues of phosalone and permethrin. The occurrence and impact of surface avoidance with respect to...
Coragen residues remains to be investigated. Further testing is needed to separate the possible effects of direct topical contact and indirect contact with residues associated with exposure vessel surfaces. This could easily be accomplished by moving spiders to clean dishes after spraying or by spraying dishes and introducing spiders afterwards, as suggested by IOBC standards (Hassan, 1985). This would help to isolate the direct topical effects of Coragen on spiders.

The effects of Coragen on spiders in the field may also differ substantially from those observed in the laboratory (Pekár, 2012, 2013). In the field, different spider species have vastly different life histories and ecological preferences and therefore may display different susceptibilities to pesticides (Bristowe, 1958; Pekár, 2012). For example, *Tibellus* are strictly active hunters and do not depend on webs for prey capture, whereas *E. ovata* often build webs or use the webs of other spiders to capture their prey (Bristowe, 1941; Agnarsson, 2002). This may put *E. ovata* at less risk of exposure from sprayed insecticides in the field because webs have been found to act as a buffer that can effectively accumulate spray droplets, essentially reducing direct topical exposure to resident spiders (Bristowe, 1958; Uetz, 1991; Pekár, 1998). In contrast, Mansour and Nentwig (1988) found that the spider *Philodromus aureolus* (Araneae: Philodromidae), a close relative of *Tibellus*, is resistant to approximately 30 pesticides. Therefore, it is possible that philodromids might be more resistant to pesticides due to intrinsic differences in physiology and biochemistry or through effective avoidance behaviours. Additional testing of the effects of Coragen on spiders in the field is required as this will provide greater insight into the impact of this product under the most realistic exposure scenario possible and thus help to further illuminate its effects on spider diversity and abundance.
4.2 Sublethal effects of diamides and hormesis in spiders

Studying the effects of pesticides on spiders at lower, sublethal dosages is important because sublethal effects can be equally problematic in ensuring the success of an IPM program (Pekár, 2013). Also, due to variability in pesticide delivery onto complex plant surfaces and combined with environmental degradation, it is expected that spiders would be more likely to encounter sublethal concentrations of a compound than acutely lethal ones in the field (Pekár, 2012, 2013). Sublethal effects are defined by Pekár (2012) as the behavioural or physiological changes in an organism following exposure to survivable levels of a chemical compound. For spiders, this may result in compromised locomotion, prey capture (e.g. web-structure and coordination while hunting), reproduction, dispersal, and defence (Pekár, 2012, 2013). In this thesis, sublethal topical exposure to Coragen was found to have no negative effects on predation of fruit flies for either of the spider genera tested. The effect of Coragen at sublethal dosages appeared to have an all or none type response in which spiders immobilized by Coragen became unresponsive and unable to feed, whereas spiders that were not immobilized by Coragen continued to successfully kill fruit fly prey. Studies by Dinter et al. (2010) demonstrated no sublethal effects of Coragen on honey bees, Apis mellifera (Hymenoptera: Apidae), and bumble bees, Bombus terrestris (Hymenoptera: Apidae). Similarly, reproduction was found to be unaffected by sublethal concentrations of chlorantraniliprole in three tephritid fruit fly species (Teixeira et al., 2009), and seven species of parasitoid wasps (Brugger et al., 2010). However, studies by Smagghe et al. (2013) found reduced reproduction in B. terrestris treated with sublethal concentrations of chlorantraniliprole. Reduced reproduction was associated with lethargic behaviour following oral intake resulting in less foraging and feeding. This was argued to be detrimental for B. terrestris fecundity in the field because it limited nutrient acquisition essential for growth and development of the colony.
Although there were no negative sublethal effects found on feeding behaviour for the two genera of spiders tested in this thesis, it is possible Coragen might be affecting other aspects of spider biology (e.g., reproduction, dispersal, defence, development, mating success, etc.). Further testing of Coragen at different sublethal concentrations and with regard to additional behavioural and physiological traits is needed.

Exposure to sublethal concentrations of pesticides may also lead to the improved performance of organisms, a phenomenon known as hormesis (Stebbing, 1982). Reported hormetic responses to organic and inorganic chemicals include improved survival, growth, and reproduction in a variety of organisms across all kingdoms (Calabrese, 2008, 2010). In spiders, the organophosphate—methamidophos, was found to improve hunting and searching efficiency of prey in *H. graminicola*, *P. amentata*, and *P. pseudoannulata* at sublethal concentrations (Toft & Jensen, 1998; Wang *et al.*, 2006b; Deng *et al.*, 2007). Similarly, low concentrations of the neonicotinoid—imidacloprid were found to stimulate predatory behaviour in *P. pseudoannulata* (Chen *et al.*, 2012). It was reasoned that attack rate increased in *P. pseudoannulata* possibly due to enhanced enzymatic activity of carboxylesterases when treated with low concentrations of imidacloprid (Chen *et al.*, 2012). In my experiments, I found that *Tibellus* treated with a sublethal dosage of Coragen killed significantly more fruit flies than untreated spiders after 4 h. A similar increase was observed in *E. ovata* after both 4 and 24 h, but the effect was not statistically significant. Although it is possible that Coragen could be having a hormeric effect on spiders at low concentrations, a causal explanation remains to be established. It is possible that increased activity and movement following introduction to the new arena may have been a contributing factor to improved hunting success. Further testing of Coragen at sublethal concentrations and on different spider species would be needed to test this hypothesis.
4.3 Chlorantraniliprole resistance and detoxification in spiders

Understanding the mechanisms by which target pests become resistant to pesticides can help prolong efficacy of newly developed pesticides and better direct future IPM practice (Cao et al., 2010; Lai et al., 2011; Sial et al., 2011). Resistance development to chlorantraniliprole first became apparent in 2011 when farmers in the Guangdong Province of China noticed the reduced efficacy of chlorantraniliprole towards *P. xylostella* (Wang & Wu, 2012). Follow up studies corroborated high levels of resistance to diamide insecticides in *P. xylostella* in Thailand, China, and the Philippines (Edralin et al., 2011; Sukonthabhirom et al., 2011; Troczka et al., 2012; Wang & Wu, 2012; Wang et al., 2013). Resistance was also found in other lepidopterous pests, including *C. rosaceana* and *S. exigua* (Lai et al., 2011; Sial et al., 2011). Pests were found to develop resistance through two primary means, increased metabolic processes resulting in enhanced detoxification (*i.e.* overexpression or upregulation of detoxification enzymes such as MFOs, esterases, and GSTs) (Cao et al., 2010; Lai et al., 2011; Sial et al., 2011;) or through the development of alterations at the RyR target site (Troczka et al., 2012; Guo et al., 2014). Studies by Sial et al. (2011) found increased esterase activity in chlorantraniliprole-resistant *C. rosaceana* larvae in laboratory tests. Similar studies by Lai et al. (2011) found both esterase and MFO levels were increased in chlorantraniliprole-resistant strains of *S. exigua*. Studies by Cao et al. (2010) reported increased esterase and GST activity induced by chlorantraniliprole in *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae. These findings suggest the potential role of enhanced detoxification enzyme activity in the metabolism of chlorantraniliprole. However, mechanisms of resistance to a particular insecticide can vary from one population to another and depending on the organism investigated (Smirle et al., 1998). More recent studies by Troczka et al. (2012) found resistance to chlorantraniliprole in *P. xylostella* to be associated with a glycine to glutamic acid substitution in the
C-terminal region of the ryanodine target site. This point mutation (G4946E) was confirmed by Guo et al. (2014) to be the most probable mode of chlorantraniliprole resistance in *P. xylostella*. Higher chlorantraniliprole resistance was associated with higher mutation frequency and lower binding affinity with the RyR target site in laboratory tests (Guo et al., 2014). Crystallographic imaging has only been developed for type I mammalian RyRs (Amador et al., 2009) and is yet to occur for that of insects, therefore, it is difficult to address exactly how mutations at insect RyRs are conferring resistance to chlorantraniliprole (*i.e.* sterically or polarly). Further testing of these mechanisms is essential in determining which mode of resistance is most likely to occur in crop pests and how to best manage it in IPM.

Owing to the limited toxicological research surrounding spiders, little is understood of the mechanisms involved in their metabolism and tolerance of pesticides. There have only been a few studies looking at the detoxification enzymes employed by spiders to metabolize toxicants. Studies by Nielsen et al. (1999) found that GST levels in *P. amentata* were only slightly induced after treatment with the pyrethroid cypermethrin. Basal levels of GST were found to be higher in both spring and autumn when the spiders were active, compared to during the winter. GSH-Px levels were also found to be highly induced by cypermethrin during spring and autumn (Nielsen et al., 1999). The levels of these enzymes in response to cypermethrin were reasoned to fluctuate depending on the season and the activity of the spiders. Studies by Chen et al. (2012) found imidacloprid significantly inhibited the activity of carboxylesterases, acetylcholinesterases, and MFOs in *P. pseudoannulata*. Similarly, carboxylesterases and acetylcholinesterases were found to be inhibited by the organophosphate dimethoate in spiders (Babczynska et al., 2006; Peng et al., 2010). Reduced levels of detoxification enzymes at high pesticide concentrations may indicate that enzymes bind with the toxin since fewer enzymes were recovered at higher pesticide
concentrations compared to controls (Nielsen et al., 1999). In my research, the synergist PBO was used to investigate the potential detoxification pathways responsible for Coragen metabolism in *E. ovata* and *Tibellus*. The inhibitory effect of PBO on oxidative detoxification enzymes (*i.e.* MFOs—cytochrome P450) has been well characterized in studies by Hodgson and Philpot (1974) as well as by Casida (1970). Studies have also shown that PBO can inhibit esterases crucial in hydrolytic detoxification (Young et al., 2006; Li et al., 2007a; Pereira et al., 2014). By studying the detoxification enzymes responsible for toxicant metabolism we can identify the pesticide chemistries that are least harmful to spiders and those which may best adhere to IPM strategies.

In my experiments on *E. ovata* and *Tibellus* I found that PBO synergized effectively with Coragen. This synergistic effect was observed in both genera of spiders. Mortality increased greatly when spiders were treated with a combination of PBO and Coragen. These findings suggest the importance of oxidative and/or hydrolytic enzymes (*e.g.*, cytochrome P450 and esterases) in the detoxification metabolism of Coragen by spiders. Similar synergistic effects have been previously observed in Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) (Jiang et al., 2012); the Asiatic stem borer, *Chilo suppressalis* (Lepidoptera: Pyralidae) (He et al., 2014); and the oriental leafworm moth, *Spodoptera litura* (Lepidoptera: Noctuidae) (Muthusamy et al., 2014). These studies indicate that in at least some cases chlorantraniliprole tolerance and ultimately resistance may be the result of cytochrome P450 and esterase activity. Studies by Salman et al. (2015) found the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae) has developed some metabolic resistance to chlorantraniliprole after being selected for resistance to the miticide acequinocyl. Resistance was determined to be the result of P450 and esterase enzyme activity determined from PBO, S-benzyl-O,O-diisopropyl phosphorothioate (IBP), and diethyl maleate (DEM) inhibition assays. Furthermore, studies by Sial and Brunner (2012) found increased toxicity of
chlorantraniliprole to the obliquebanded leafroller, *C. rosaceana*, when treated with the esterase inhibitor S,S,S-tributylphosphoro trithioate (DEF). PBO resulted in the next greatest increase in toxicity to chlorantraniliprole (Sial & Brunner, 2012). Therefore, both oxidases and esterases have been shown to be involved in the detoxification metabolism of chlorantraniliprole in a variety of arthropods. The results of my experiments corroborate these findings. However, further testing with more specific enzyme inhibitors (e.g. the esterase inhibitor DEF) needs to be conducted to identify the primary mode of detoxification in spiders. Further testing on other species of spiders would also help to reinforce the generality of these results.

### 4.4 Conclusions

Spider toxicology is still in its infancy, with less than a couple of hundred spider species and a handful of pesticides rigorously tested in field and laboratory studies (Theiling & Croft, 1988; Pekár, 2012). Considering there are more than 45 000 spider species (World Spider Catalog, 2015) and thousands of pesticides registered globally, there are still many combinations of representative spider species and pesticides remaining to be examined. Most toxicological research on spiders has focused on field studies where the effects of pesticides on individual spiders may be concealed by factors such as migration, pesticide degradation, and avoidance of exposure (Theiling & Croft, 1988; Markó *et al.*, 2009; Pekár, 2012; Yang *et al.*, 2014). Field studies focus on more realistic conditions than laboratory assays, but do not provide a complete understanding of the direct effects of pesticides. The field of spider toxicology would benefit from more studies under laboratory conditions, where routes of exposure can be isolated, and lethal and sublethal effects observed. Studies of this nature may help to better direct pesticide chemistries and application methods to
enhance selectivity for pests while minimizing harm to spiders and other beneficial arthropod communities.

My project is one of the few to study the lethal and sublethal effects of chlorantraniliprole on spiders as well as the potential routes of its metabolism. Coragen was found to be moderately harmful (51–75% mortality) to harmful (>75% mortality) according to IOBC standards (Hassan, 1985) for both *Enoplognata* and *Tibellus* at rates and concentrations comparable to those applied in the field. Exposure in the field may differ substantially from that in the lab and thus, more thorough investigation of chlorantraniliprole on spiders in the field is needed. Although both the spider genera tested are known to migrate into field crops at some point during their life cycles, they are not true agrobionts and predominantly inhabit field margins. Therefore, future research regarding Coragen drift and its impact on activity of beneficial arthropods in field margins is imperative.

Sublethal concentrations of Coragen demonstrated no negative impact on feeding behaviour for both spider genera tested, however, a potential hormetic effect may have been observed since treated spiders were found to kill more fruit flies on average than untreated spiders. A wide array of concentrations and behavioural/physiological traits still need to be investigated to gain a full understanding of the sublethal effects of Coragen on spiders. Experiments involving PBO revealed high dependence of both spider genera on oxidative and/or hydrolytic detoxification pathways in the metabolism of Coragen. Inhibition of MFOs and esterases with PBO resulted in greatly increased mortality in both spider genera. Future studies to determine which pathway is primarily responsible for Coragen metabolism is essential in understanding an important underlying basis for resistance. Spiders are a vital group of predatory arthropods present in various ecosystems. Their role in moderating pest populations is undisputable and has been shown to be negatively influenced by anthropogenic factors, especially pesticides. Preserving spider populations and minimizing our
impact on them is crucial for optimizing IPM strategies and in maintaining the future success of agricultural practice as a whole.
LITERATURE CITED


DuPont™ Altacor® insecticide. 2013. Main label.


DuPont™ Coragen® Insect Control. 2010. Supplemental label.


Appendix 1

FRUIT FLY REARING

Fruit flies, *Drosophila* spp. (Diptera: Drosophilidae), were used to feed the spiders and provided a desirable prey item for *E. ovata* and *Tibellus* spp. in the sublethal experiments. Fruit flies were reared in 100 × 25 mm plastic Petri dishes (Fisher Scientific, Ottawa, Canada) on a diet consisting of a mixture of cornmeal (125 g), sugar (white) (200 g), nutritional yeast (70 g), water (2.8 L), 95% ethanol (33.3 mL), and agar (45 g). Propionic acid (17.7 mL) and methyl paraben (3.3 g) were used to stabilize the mixture and provide antifungal/antibacterial properties to the diets. The prepared diets had the dual purpose of providing food for the fruit flies as well as substrate for ovipositing. When 30–50 adult fruit flies emerged in the rearing diets, they were released and used to supplement or start new colonies. Colonies were maintained in plastic enclosures (23 × 23 × 26 cm) and had their diets replaced every two to three days. Removed diets were sealed and stored for incubation at standard ambient lab conditions (21.5 ± 1°C, 50 ±8 % RH). Deionized water was provided on cotton for moisture and replenished weekly.
Appendix 2

SPRAY TOWER CALIBRATION

The miniature spray tower was an essential piece of equipment used for all experiments in this thesis (Figure A2.1). The tower was used to eject various solutions (deionized water, Coragen, and PBO) as aerosols in order to topically treat spiders. Dispensed solution volumes were isometric at 1 mL and thus, testing different rates required solution concentrations to be varied. However, knowing the concentration of a solution and the volume at which it was applied only gives a theoretical understanding of how much solution might be actually reaching the spiders. To make more realistic and accurate inferences, extrapolations, and comparisons, especially when considering field applied rates, it was imperative to determine the precise output of the spray tower. Calibration tests of the spray tower were conducted to investigate its deposition rate and output efficiency. This was done by comparing the weights of test materials before and after treatment with 1 mL of solution. The solutions tested include deionized water, acetone, 70% ethanol, and 19:1 acetone + olive oil. Only the results from the deionized water tests were included as no observable or measurable differences were found for the other solutions.
Initial tests investigated the overall output of the tower. Paper towel (n=5), quartered paper towel (n=10), and the combined weight of the full set-up (paper towel, 60 × 15 mm Pyrex® Petri dish, and filter paper) (n=20) were analyzed before and after 1 mL applications of deionized water (Table A2.1).
Table A2.1. Total spray tower output and deposition.

<table>
<thead>
<tr>
<th>Volume of DI water applied (mL)</th>
<th>Sample size (n)</th>
<th>Mean ± SE weights of test materials</th>
<th>Mean ± SE difference/deposition (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dry weight (g)</td>
<td>Wet weight (g)</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>2.09 ± 0.01</td>
<td>2.33 ± 0.01</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>0.509 ± 0.006</td>
<td>0.714 ± 0.006</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>17.2 ± 0.2</td>
<td>17.3 ± 0.2</td>
</tr>
</tbody>
</table>

\[
\text{Average } \% \text{ recovery} = \frac{\text{solvent deposited (mg)}}{\text{solvent dispensed (mg)}} \times 100
\]

\[
= \frac{((241 + 204 + 180)/3)}{1000} \times 100
\]

\[
= 20.8 \pm 0.4 \%
\]

The total percentage of deionized water recovered at the base of the spray tower following 1 mL applications ranged from 18–24%. The average total output was 20.8 ± 0.4%. The total output area was 63.617 cm\(^2\). Following this, the amount of solution recovered by the 60 \(\times\) 15 mm test dishes (Area = 28.274 cm\(^2\)) was investigated. This involved looking at the combined weight of the test dish and filter paper before and after treatment with 1 mL applications of deionized water (Table A2.2).

Table A2.2. Deposition for a 60 \(\times\) 15 mm Pyrex\textsuperscript{®} Petri dish.

<table>
<thead>
<tr>
<th>Test</th>
<th>Volume of DI water applied (mL)</th>
<th>Sample size (n)</th>
<th>Mean ± SE weights of test materials</th>
<th>Mean ± SE difference/deposition (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dry weight (g)</td>
<td>Wet weight (g)</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>5</td>
<td>17.0 ± 0.3</td>
<td>17.1 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>5</td>
<td>16.7 ± 0.6</td>
<td>16.7 ± 0.6</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>10</td>
<td>16.6 ± 0.4</td>
<td>16.6 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>20</td>
<td>16.7 ± 0.2</td>
<td>16.7 ± 0.2</td>
</tr>
</tbody>
</table>

\[
\text{Average } \% \text{ recovery} = \frac{\text{solvent deposited (mg)}}{\text{solvent dispensed (mg)}} \times 100
\]

\[
= \frac{((16 + 19 + 28 + 23)/4)}{1000} \times 100
\]

\[
= 2.14 \pm 0.09 \%
\]
The deposition rate in the test dishes ranged from 1.6–2.8%. The average deposition rate in the test dishes was 2.14 ± 0.09%. Losses in these calibration tests were likely due to volatilization, solution intercepted/trapped on the interior walls of the spray tower, and/or solution lost in the area around the test dish.
Appendix 3

THEORETICAL LD$_{50}$ (MG INSECTICIDE PER KG OF BODY WEIGHT) FOR TIBELLUS

Adult female Tibellus spp. were found to be susceptible to Coragen at rates comparable to those recommended for field use by DuPont (0.563 and 1.125 mg mL$^{-1}$). The average mortalities for the 2013 and 2014 field seasons were 42% at 0.563 mg mL$^{-1}$ and 79% at 1.125 mg mL$^{-1}$. A LD$_{50}$ concentration of about 0.75 mg mL$^{-1}$ was estimated with consideration of the acute toxicity trends of these dose-response data. Dispensed solution volumes were isometric at 1 mL. Deposition rate onto the 60 × 15 mm Pyrex® test dishes (Area = 28.274 cm$^2$) was corrected by 2.14% (See Appendix 2: Spray Tower Calibration).

\[
C = \text{Concentration (mg/mL)}
\]
\[
V = \text{Volume dispensed (mL)}
\]
\[
CF = \text{Correction factor}
\]

\[
Deposition \text{ at } LD_{50} = C \times V \times CF
\]
\[
= (0.75 \text{ mg/mL}) \times (1 \text{ mL}) \times (2.14\%)
\]
\[
= 0.01605 \text{ mg}
\]

\[
\text{Application Rate} = \frac{Deposition}{\text{Area of dish}}
\]
\[
= \frac{0.01605 \text{ mg}}{2827.4 \text{ mm}^2}
\]
\[
= 5.6766 \times 10^{-6} \text{ mg mm}^{-2}
\]

Therefore, a total of 0.01605 mg of Coragen would be deposited onto the filter paper of a test dish at a rate of $5.6766 \times 10^{-6}$ mg mm$^{-2}$ (or $5.6766 \times 10^{-3}$ µg mm$^{-2}$). In order to determine how much of this application rate would be intercepted by the spiders, their surface area was estimated (Table A3.1). Surface area was estimated from an aerial photograph of a representative adult female Tibellus on graphing paper.
Table A3.1. Estimated surface area of adult female *Tibellus*.

<table>
<thead>
<tr>
<th>Body Part</th>
<th>Approximate Length (mm)</th>
<th>Approximate Width (mm)</th>
<th>Estimated Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legs (×8)</td>
<td>12</td>
<td>0.75</td>
<td>72</td>
</tr>
<tr>
<td>Abdomen</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Cephalothorax</td>
<td>4</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Total Estimated Area:</strong> 89 mm²</td>
</tr>
</tbody>
</table>

Multiplying the application rate by the estimated spider surface area provides an approximate value of the amount of Coragen intercepted by the spiders (≈0.5 µg). This is presumably an overestimate because of the roughness in calculating the complicated surface area of the spiders. The average wet weight of adult female *Tibellus* was determined to be about 17.0 ± 0.1 mg (n=20). Therefore, about 0.5 µg of Coragen is killing ≥50% of adult female *Tibellus* spp. weighing 17.0 mg. The estimated LD₅₀ would be about 29 mg kg⁻¹.
Appendix 4
SEXING SPIDERS BY PEDIPALPS

Figure A4.1. Illustration of male and female spider pedipalps (palps).
Source: Daily Arachnophile.
**Table A5.1. Physical and chemical properties of chlorantraniliprole (CAS # = 500008-45-7) (3-Bromo-N-[4-chloro-2methyl-6-[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide). Source: US EPA.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metling point/range (°C)</td>
<td>200-202 (95.9%)/208–210 (99.2%)</td>
</tr>
<tr>
<td>pH</td>
<td>5.77 ± 0.087 at 20°C</td>
</tr>
<tr>
<td>Relative Density</td>
<td>1.5189 (95.9%)/1.507 (99.2%) at 20°C</td>
</tr>
<tr>
<td>Water solubility (20°C)</td>
<td>DI water (1.023 mg/mL), pH 4 (0.972 mg/mL), pH 7 (0.880 mg/mL), pH 9 (0.971 mg/mL)</td>
</tr>
<tr>
<td>Solvent solubility (20°C)</td>
<td>Acetone (3.446 ± 0.172 g/L), Acetonitrile (0.711 ± 0.072 g/L), Ethyl Acetate (1.144 ± 0.046 g/L), Dichloromethane (2.476 ± 0.058 g/L)</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
</tr>
</tbody>
</table>