BLUEBERRY SPANWORM, *ITAME ARGILLACEARIA* (PACKARD) AND BUMBLE BEE, *BOMBUS IMPATIENS* (CRESSON) SUSCEPTIBILITY TO NEW BIORATIONAL INSECTICIDES

by

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DEDICATION PAGE

To a man whose sense of humour persevered and infected all of us even through times of great sadness.

I dedicate this Thesis to my late uncle, Emmanuel Gabriel David, who passed away on December 5 2008. Thank you for all your love and laughs throughout my childhood and adult years.

I wish we had more time together.

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Abstract

Biological and cultural control tactics are available for many agricultural pests but insecticides still play an important role in the rapid reduction of pest incidence when damage reaches economic levels. Laboratory and field toxicities of the reduced-risk products spinetoram and flubendiamide to *Itame argillacearia* (blueberry spanworm) was compared to deltamethrin, a conventional synthetic pyrethroid insecticide. In laboratory experiments, *I. argillacearia* larvae were highly susceptible to spinetoram and flubendiamide, and efficacy in the field was comparable to that of deltamethrin. Lethal and sublethal effects of the biopesticide formulations of *Beauveria bassiana* and *Bacillus subtilis*, and spirotetramat, a new tetramic acid insecticide, to bumble bees, *Bombus impatiens*, were also assessed. When ingested, field rates of spirotetramat caused high mortality after a week, and *B. subtilis* significantly reduced drone production. Field rates of spirotetramat, when applied topically, reduced drone production, but drone production varied following topical treatments of either biopesticide.

List of Abbreviations Used

A.I. or a.i.:	active ingredient
cm:	centimeter (1 cm = 1 x 10^{-2} m)
CFU:	colony forming units
C.I.:	confidence interval
DAT:	days after treatment
df:	degrees of freedom
et al:	and others
F:	F-test statistic
g:	gram
h:	hour
ha:	hectare (1 ha = 10,000m^2)
Inc.:	incorporated
IPM:	integrated pest management
kg:	kilogram (1 kg = 1 x 10^3 g)
km:	kilometer (1 km = 1 x 10^3 m)
kpa:	kilopascals
L:	liter
L:D:	light: dark
LC50:	lethal concentration that kills 50 % of a population
LD50:	lethal dose that kills 50 % of a population
LRFC:	least recommended field rate
m:	meter
mg:	milligram (1mg = 1 x 10^{-3} g)
mm:	millimeter (1 mm = 1 x 10^{-3} m)
mL:	milliliter (1 mL = 1 x 10^{-3} L)
MRFC:	maximum recommended field rate
n:	sample size
NSAC:	Nova Scotia Agricultural College
P:	P – value
ppm:	parts per million (1 ppm = 1 mg L^{-1})
R^2:	regression coefficient of determination
RH:	relative humidity
SEM:	standard error of the mean
α:	level of significance
^oC:	degree Celsius
™:	trademarked
®:	registered
µg:	micrograms (1 µg = 1 x 10^{-6} g)
χ^2:	chi-square

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CHAPTER ONE: INTRODUCTION

1.1: Wild Blueberries

Vaccinium angustifolium Aiton. (wild blueberry) is an important horticultural crop grown mostly in northeastern North America (Prior *et al.*, 1998). The largest production regions are the state of Maine, Québec and the Atlantic provinces. In 2008, the total farmgate value of wild blueberries in Canada was $115 million (Bussmann, 2008). Blueberries, including highbush blueberries grown primarily in BC and Ontario, are the number one horticultural crop in Canada in terms of acreage and value (Robichaud, 2006).

Vaccinium angustifolium is a small shrub that grows to about 35 cm high and is often one of the first plants to colonize freshly disturbed areas (Kinsman, 1986). The plant can reproduce by seed but spreads mainly by clonal propagation through a network of underground rhizomes (Barker & Collins, 1963; Hall *et al.*, 1972). There are slight morphological and physiological differences between clones (Hall *et al.*, 1979). Another species of lowbush blueberry, *Vaccinuim myrtiloides* Michx., often cohabits blueberry fields (Vander Kloet, 1981), although *V. angustifolium* is more valued in crop production.

Vaccinium angustifolium produces small dark blue fruit. Fruit set of wild blueberries is facilitated with cross-pollination of flowers, which is achieved mainly through bees and other pollinating insects (Aalders & Hall, 1961). In large production systems, it is thought that native pollinators are usually not abundant enough to provide adequate pollination and harvestable yields, and managed bees are usually used as a

supplementary pollination force (Javorek *et al.*, 2002)(Ismial, 1987). The most widely used bee for pollination in lowbush blueberry is the European honey bee, *Apis mellifera* L.

Vaccinium angustifolium is managed on a two-year cycle in most regions (Barker *et al.*, 1964). Large growers usually alternate fields so that half of their fields will be in the vegetative "sprout" phase, while the other half will be in the harvestable "crop" phase each year. After a field of blueberries has yielded fruit, it is pruned back to the ground either by burning or mowing to maintain the highest berry yield (Eaton & McIsaac, 1997). Traditionally, burning was the most widely practiced pruning method but with rising costs of oil and environmental concerns regarding CO_2 emissions, mowing is now mostly used (Eaton & McIsaac, 1997). Burning likely provided control of many weeds, diseases and insects, and the switch to mowing has resulted in increased pest problems (Kinsman, 1993). Important insect pests of blueberries include *Itame argillacearia* Packard (blueberry spanworm), *Altica sativa* Malloch (blueberry flea beetle) and *Rhagoletis mendax* Curran (blueberry fruit fly) with spanworm being the primary defoliator in many parts of Nova Scotia.

1.2: *Itame argillacearia* (Blueberry Spanworm)

1.2.1 Biology

Itame argillacearia (Lepidoptera: Geometridae) in Canada ranges from Quebec to Nova Scotia, and in the United States from Maine to West Virginia (Wagner *et al.*, 2001). This insect has also been found in highbush blueberries and cranberries (Turner

& Liburd, 2007; , 2009), but most of the known biology and ecology of *I. argillacearia* is based upon work in *V. angustifolium*. The insect overwinters as an egg at the base of blueberry plants in leaf litter (Drummond 2000). Eggs are pink to green in colour, about 1 mm in diameter, and usually hatch from late May to early June in Nova Scotia (Crozier 1995) (Fig. 1a). Egg hatch generally coincides with blueberry bud break (Drummond & Groden, 2000). First instar *I. argillacearia* larvae appear in early June and are tan or grey, with a series of black spots along the dorsal side. Older larvae are orange coloured, with black spots running in lines along the body, sometimes looking like a continuous stripe (Crozier, 1995) (Fig. 1b). There are typically a total of five instars[1].

Larvae walk in a looping fashion by contracting the posterior of their body towards their head in a loop, and then extending the anterior end of their body to move forward (Crozier 1995). Fourth instar larvae can grow up to 20 mm in length (Crozier, 1995). Larvae can cause severe defoliation in crop fields of blueberries and can cause significant damage to emerging shoots in sprout fields. *Itame argillacearia* larvae feed on leaves, flower buds and blossoms (Drummond & Groden, 2000; Booth *et al.*, 2007). Feeding is mostly done at night as larvae tend to inhabit lower levels of the plant in the day (Crozier, 1995; Drummond *et al.*, 2008). In sprout fields, *I. argillacearia* feeds upon new growth, severely compromising later development of plant shoots (Crozier, 1995). In Nova Scotia, *I. argillacearia* larvae usually feed until late June or early July, after which they fall to the ground for pupation (Fig. 1c). Adult moths usually emerge in late July and mate shortly thereafter (Crozier 1995).

[1] Judith Collins. Research Associate, University of Maine, USA. Personal Communication. January 21, 2009

Figure 1 Life stages of *Itame argillacearia*. [2] picture courtesy of Jim des Rivieres www.moths.ca

Itame argillacearia adults are gray to brown coloured with females having darks spots on wings, and male wings uniform in colour (Crozier, 1995) (Fig. 1d). The wingspan ranges from 23-29 mm (Crozier, 1995). Flight ranges of the adult moths are still unknown. Alford and Diehl (1985) found that adult females periodically release sex-pheromones to "call out" to males and that most calling happens at dawn during the first few hours of light. Adults mate in late July to early August and mated females lay eggs at the base of the blueberry plant, which do not hatch until the following year (Crozier, 1995).

1.2.2 Pest Status and Control of *I. argillacearia*

Larvae of *I. argillacearia* can be a major pest of wild blueberry agroecosystems (Crozier, 1995). The government of Canada has identified that control options for *I. argillacearia* are a key issue in wild blueberry production (Bussmann, 2008).

There are a number of natural enemies that attack *I. argillacearia.* Predators include the beetles *Harpalus rufipes* De Geer (Coleoptera: Carabidae), which are found in May and again in August and September (the same time *I. argillacearia* is in the egg stage), and *Calosoma calidum* Fabricius (Coleoptera: Carabidae), which are found in June and July when *I. argillacearia* is in the larval stages (Drummond *et al.*, 2008). Opiliones (Arachnidia) may also be important predators (Drummond *et al.*, 2008). Several species of tachinid parasitoids (Diptera) (Drummond & Groden, 2000) and parasitic wasps (Diapriidae, Chalcidoidea, Cynipoidea, Proctotrupoidea) also are known to attack *I. argillacearia* (Karem *et al.*, 2006). Naturally occurring pathogens, such as *Beauveria bassiana* [Balsamo-Crivelli] Vuillemin and *Metarhizium anisopliae*

Metschinkoff also exist and can infect *I. argillacearia*. *Bacillus thuringiensis* Berliner is also present and the toxin produced by this bacteria can cause mortality in *I. argillacearia* (Drummond *et al.*, 2008).

Unfortunately, these natural enemies often inadequately regulate high populations of *I. argillacearia* and with the exception of Dipel® (Valent BioSciences Inc.), a formulation of *B. thuringiensis* that is registered for use in wild blueberry, there are no natural control agents available for purchase and use in augmentative biological control. Hard burning was an effective form of pest control, but as previously mentioned, there has been a shift to mowing instead of burning (Kinsman, 1993). No other effective cultural, physical, or semiochemical control tactics have as of yet been developed.

Chemical insecticides are useful tools for wild blueberry IPM, and most growers are reliant on chemicals for *I. argillacearia* control. However, most registered products for *I. argillacearia* control are organophosphorous (OP), carbamate and pyrethroid insecticides, which are broad-spectrum nerve poisons, although the pyrethroids have relatively low mammalian toxicity. Chemical controls for *I. argillacearia* have included (past and/or present): Dylox 420L®(trichlorfon), Decis 5 EC® (deltamethrin), Sevin XLR Plus® (carbaryl) and Imidan 50WP® (phosmet) (Delbridge & Rogers, 2008). With the exception of Dylox, all products remain registered for control of *I. argillacearia* on blueberries. Due to deregistration of OP insecticides, a public that demands safer and more ecologically responsible food production, and increasingly stringent IPM requirements of major international markets, the wild blueberry industry requires integration of new effective, yet safer insecticides with lower toxicity to pollinators.

Thus, there is a strong impetus for research into novel biorational insecticides for *I. argillacearia* management.

1.3: Biorational Insecticides in Integrated Pest Management

1.3.1 Early Insecticide Use

Synthesized insecticide use in agriculture began shortly after the Second World War with the introduction of DDT, an organochlorine insecticide. This ushered in the use of chemical control as the main method of combating arthropod pests in agriculture and other insecticide classes – mainly the organophosphorous (OP) insecticides (parathion in 1948), carbamates (carbaryl in 1958) and synthetic pyrethroid insecticides (permethrin and fenpopathrin in the 1970s) – were soon discovered or developed as chemical control approaches gained momentum (Casida & Quistad, 1998). Although effective, DDT posed a serious hazard to wildlife, as brought to attention in Rachel Carson's *Silent Spring*, and along with most other organochlorines insecticides, has been banned in North America. OP and carbamate insecticides have significant human health and environmental risks surrounding their use, and are noted for their relatively high toxicity to many animals, especially bees (Davignon *et al.*, 1965; Schafer, 1972; Zinkl *et al.*, 1977; Fest, 1982; Anonymous, 1986; Nicolaus & Lee, 1999; Farahat *et al.*, 2003). OPs, and carbamates are also harmful to natural enemies (Brunner *et al.*, 2001; Deng *et al.*, 2006), and other wildlife (Kurtz & Studholme, 1974; Kristoff *et al.*, 2010). Consequently, many of the active ingredients in these insecticide classes are being phased out of production in North America. Pyrethroid insecticides continue to be used

in many agricultural systems, but may exhibit adverse effects on birds (Hill, 1985), fish (Köprücü & AydIn, 2004), bees (Benedek, 1983; Charnetski, 1988; Atkins, 1992) and non-target arthropods (Hill, 1985; Wiles & Jepson, 1992).

1.3.2 Biorational Insecticides

Modern consumers are demanding safer food and food production practices that involve less use of broad spectrum neurotoxins in crop protection. Biorational pest control agents are synthetic or natural insecticides that provide control of pest species but pose less risk to humans, non-target species and the environment (Djerassi *et al.*, 1974). These include compounds that target specific developmental processes in insects (e.g. chitin synthesis inhibitors, juvenile hormone mimics, ecdysone agonists), biopesticides (e.g. *B. thuringiensis*, *B. bassiana*, *Saccharopolyspora spinosa* Mertz and Yao) and several compounds that selectively act on biochemical sites in insects to cause mortality. Many of these pest management alternatives may have potential for control of *I. argillacearia* in wild blueberry. Brief descriptions of the different active ingredients and insecticide classes explored in this thesis follow.

Chitin synthesis inhibitors, juvenile hormone mimics and ecdysone agonists are all insect growth regulating compounds and there are a number of products available (e.g. novaluron, methoxyfenozide and pyriproxifen). These all show high safety to mammals but are not selective among insects (Declercq *et al.*, 1995; Cutler *et al.*, 2006), although methoxyfenozide does show a relatively high degree of safety to non-target arthropods (Baur *et al.*, 2003; Kim *et al.*, 2006)

The neonicotinoids (e.g. imidacloprid, acetamiprid) are the most important and widely used insecticides developed over the past two decades. They act on the nicotinic acetylcholine receptors in insects and have a high degree of safety towards mammals, but there are concerns about their safety to bees (Tomizawa & Casida, 2003). The neonicotinoids are mostly effective against sucking pests, some lepidopteran and coleopteran pests (Mizell & Sconyers, 1992; McCullough & Smitley, 1995; Yue *et al.*, 2003).

Spinosyns are an insecticide class derived as a metabolic by-product of the soil dwelling actinomycete *S. spinosa.* They are highly toxic to most insects but pose reduced risk to mammals, birds, and fish (Thompson *et al.*, 2000; Salgado & Sparks, 2005). Spinosyns A and D are the active ingredients in spinosad (Waldron *et al.*, 2000). Spinosad affects nicotinic acetylcholine and gamma aminobutyric acid (GABA) receptors in the insect central nervous system, although at different sites than other insecticides (Salgado, 1998). Spinosad is generally not considered very toxic to bees at field rates (Mayes *et al.*, 2003; Spencer *et al.*, 2003; Bailey *et al.*, 2005) but, at high concentrations it may compromise bee development (Kaneshi, 2000; Miles, 2003; Morandin, 2005). Spinosyns J and L make up the active ingredient for spinetoram (Dripps *et al.*, 2008), a 'second generation' spinosyn with higher insecticidal potency but with low hazard to mammals (Anonymous, 2008). Spinetoram does pose a risk to honey bees and other beneficial arthropods when wet, but is of low risk as a dry residue (Anonymous, 2008).

The phthalic acid diamides are a new class of compound that have demonstrated very good activity against lepidopteran pests through ingestion (Tohnishi, 2005),

affecting ryanodine-sensitive intracellular calcium release channels (ryanodine receptors, RyR) in insects (Luemmen, 2005). Phthalic acid diamides have no effect on the ryanodine1 binding receptors in mammals, suggesting high insect specificity (Ebbinghaus-Kintscher *et al.*, 2006; Kato *et al.*, 2009). Flubendiamide is a phthalic acid diamide that has low acute toxicity to honey bees, and moderate oral toxicity to mammals and birds at 2000 mg a.i. per kg (Hall, 2007).

Beauveria bassiana is a naturally occurring soil fungus that adheres to insect cuticle and infects through the cuticle by producing toxins that dissolve and break down chitin (Lefebvre, 1934), as well as through the non-sclerotized parts such as intersegmental membranes (Steinhaus, 1949). The fungus grows inside the insect on fat bodies and the mycelium eventually infects all parts of the insect and penetrates through the cuticle, killing the insect and covering the remains in white hyphae (Lefebvre, 1934). *Beauveria bassiana* can infect all insects to some degree but successful infection depends on suitable temperature and humidity (Steinhaus, 1949). *Beauveria bassiana* mildly affects *A. mellifera,* but has a stronger effect on *Bombus terrestris* Linnaeus (Alves *et al.*, 1996; Hokkanen *et al.*, 2003).

The tetramic acids (e.g. spirotetramat) are a novel insecticide class used to control sucking insects. They act through inhibition of lipid synthesis, acting mainly on juveniles, but also affecting adult fecundity. Spirotetramat has been shown to be very effective for a wide variety of sucking pests, such as aphids, thrips, scales, mealy bugs and whiteflies (Bruck *et al.*, 2009). Tests on beneficial arthropods demonstrate that

spirotetramat has reasonable safety to ladybird beetles, predatory mites, and spiders (Maus, 2008).

Bacillus subtilis (Ehrenberg) Cohn is a gram positive soil dwelling bacterium that has fungicidal activity (Nakano & Zuber, 1998; Mantecon, 2008). Although not an insecticide, through its use in pest management non-target insects, including pollinators may be at risk. *Bacillus subtilis* produces many broad spectrum anti-biotics, and as a result, this microbe has been formulated into sprays that are now used to control many fungal pathogens in agroecosystems (Gueldner *et al.*, 1988; Elizabeth & Jo, 1999). It is used for control of the *Botrytis* spp. fungal pathogen, which causes botrytis floral blight in blueberries (Bernard *et al.*, 1998; Elad & Stewart, 2004).

1.4: Pollination of Wild Blueberries

1.4.1 Wild Blueberry Pollination and Bees

Cross pollination by insects, primarily bees, is critical for wild blueberry fruit set (Wood, 1961; Kinsman, 1986; Drummond & Stubbs, 2003). Managed bees, especially the honey bee, *A. mellifera*, are widely used for crop pollination. However, growers are becoming increasingly interested in the use of non-*Apis* managed pollinators such as the bumble bee (*B. impatiens*) and the alfalfa leafcutting bee (*Megachile rotundata* Fabricius), along with promoting the contribution of native pollinators. Growers are also very keen to use insecticides that pose reduced risk to bees. This is particularly true when attempting to control *I. argillacearia* since their peak populations often coincide with bloom of wild blueberries (approximately early June), meaning there is increased

risk of exposing bees to insecticides. Current insecticides used for the control of *I. argillacearia* exhibit varying levels of toxicity to bees. Some microbial control agents that have pest management potential in wild blueberry also have shown some toxicity to bees (Mommaerts *et al.*, 2009). In addition to being deleterious on their own, sublethal concentrations of insecticides can exacerbate problems in bees with compromised health due to pathogens and parasites (Alaux *et al.*, 2010) and insecticides may also be a contributing stressor to colony collapse disorder (CCD) (Williams *et al.*, 2010)(Alston *et al.*, 2007; Abbott *et al.*, 2008).

1.4.2 *Bombus impatiens* for Wild Blueberry Pollination

Concerns over honey bee health and subsequent uncertainty in availability of honey bee hives for pollination means that growers are considering alternative bee species to provide pollination services. *Bombus impatiens* is a commercially available bumble bee that is native to eastern North America (Finnamore & Neary, 1978) and has become established in Nova Scotia (Laverty & Harder, 1988). Bumble bees were domesticated in the 1970s and commercial rearing started around 1990 (Plowright & Jay, 1966; Velthuis & van Doorn, 2006). *Bombus impatiens* is a social bee that forms colonies containing a queen, workers, and drones. Development to the adult stages takes approximately 12, 16 and 22 days for workers, drones and queens, respectively (Alford, 1975). Commercial colonies can produce anywhere from 300 - 500 workers (Plowright & Laverty, 1987).

Being native to eastern North America, *B. impatiens* is a very effective pollinator of many plants native to the region, including *V. angustifolium* (Heinrich, 1979). Using

"buzz pollination" (Buchmann, 1983), bumble bees pollinate blueberry flowers more efficiently than almost any other wild bee, and may transfer four-fold more blueberry pollen per visit than honey bees (Javorek et al. 2002). They are also more faithful to blueberry flowers than other flowers in the field (Stubbs & Drummond, 1997). As such, they are a more effective pollinator of blueberry fields than honey bees on a per flower visit basis (Free, 1993). Although wild bumble bees, including *B. impatiens*, commonly overwinter in or around blueberry fields, commercial colonies must be purchased each year.

Prior to registration in Canada, insecticides usually undergo toxicity testing on all target species and many important non-target organisms. Bees are considered non-target beneficial insects and data on the toxicity of any insecticides to *A. mellifera* are required. However, many bee species can be present in agroecosystems, and, much like the pest insects of these systems, knowledge of the susceptibility of the multiple bee species is desirable to ensure appropriate IPM programs can be developed. Broad recommendations of pollinator safety based on toxicity assessments of one or two bee species should be avoided as there can be great differences in susceptibility among bee species (Johansen, 1972; Tasei, 2002; Devillers *et al.*, 2003; Dobrynin & Colombo, 2007)

There is limited information available on the susceptibility of *B. impatiens* to insecticides (Tasei, 2002), particularly products used in wild blueberry. Most of the toxicology work done on bumble bees up until now, has been done on the European bumble bee *B. terrestris* (Tasei, 2002). *Bombus impatiens* is susceptible to dry, non-irrigated residues of imidacloprid applied at rates registered for control of turf-feeding

insects (Gels *et al.*, 2002). However, *B. impatiens* survival is not negatively affected by imidacloprid granules and imidacloprid sprays at field rates when there is post-treatment irrigation (Gels *et al.*, 2002). *Bombus impatiens* did exhibit mildly negative sub-lethal effects when exposed to field rates of spinosad in the lab (Morandin, 2005) and it was recommended that higher tier testing be required by regulatory authorities. There is uncertainty concerning the susceptibility of *B. impatiens* to several reduced risk insecticides and bio-pesticides, to which they may be exposed in future *I. argillacearia* management.

1.5: Objectives and Hypotheses

Itame argillacearia is a major pest of wild blueberry and, currently, insecticides are still the most economically feasible method of control. However, wild blueberry bloom (and subsequent bee pollination) and *I. argillacearia* outbreaks occur at the same time, thus making it difficult to control this pest without harming pollinators. Therefore, insecticides that offer reduced risk to pollinators are required. There is a lack of data on susceptibility to *B. impatiens* for many of these new reduced risk products. Thus, the following broad objectives were determined:

1) To evaluate the toxicity of new bio-rational insecticide alternatives to *I. argillacearia* in the laboratory and their efficacy for *I. argillacearia* management in the field.

2) To evaluate acute toxicity, and possible sub-lethal effects, of currently registered pesticides and bio-pesticides on *B. impatiens*.

Within these objectives, I hypothesize that the tested biorational products will have low LC50 values to *I. argillacearia* and that the LC50 values of these compounds to *I. argillacearia* will be relatively consistent across two populations from Nova Scotia and New Brunswick. I also expect that the new insecticides will show a high level of insect control. When assessing effects of currently registered pesticides and bio-pesticides on *B. impatiens*, I hypothesize that the tested compounds will have variable effects on *B. impatiens* depending on their intrinsic toxicity and route of exposure.

CHAPTER TWO: TOXICITY OF BIORATIONAL INSECTICIDES TO BLUEBERRY SPANWORM (*ITAME ARGILLACEARIA* LEPIDOPTERA: GEOMETRIDAE).

2.1 Introduction:

Wild blueberry, *Vaccinium angustifolium* Aiton., is commercially produced mainly in northeastern North America. It is grown on a two year schedule, with a year of vegetative growth followed by a fruit producing year. After fruit harvest plants are pruned by mowing or burning (Eaton & McIsaac, 1997). The largest production regions are the state of Maine, and the provinces of Nova Scotia, Québec, New Brunswick and Prince Edward Island in Atlantic Canada. In 2008, the total farmgate value of wild blueberries in Canada was $115 million (Bussmann, 2008), making it one of the most valuable horticultural commodities in the country.

The blueberry spanworm, *Itame argillacearia* Packard (Lepidoptera: Geometridae), is a major defoliator of lowbush blueberry, attacking emerging shoots and mature plants. *Itame argillacearia* larvae cause damage by feeding on new growth, leaves, flower buds and blossoms (Drummond & Groden, 2000; Booth *et al.*, 2007). In Nova Scotia, overwintering eggs usually hatch in late May and developing larvae may feed until late June or early July, after which they pupate in the ground. Adult moths emerge in late July and mate shortly thereafter with eggs being laid in the leaf litter to overwinter (Crozier, 1995). In the past, burn-pruning suppressed many *I. argillacearia* populations, presumably destroying over-wintering stages (Kinsman, 1993). However, with increased public concern of environmental pollution from burning and rising fuel costs, many growers have switched to pruning by mowing, resulting in an increase in the

incidence of pest outbreaks (such as *I. argillacearia*) in some growing regions (Crozier, 1995).

Natural predators and parasites do exist for *I. argillacearia,* and the pest is also susceptible to infection from entomopathogens (Drummond & Groden, 2000). However, natural enemies alone may not be present in large enough numbers to provide economic control and few effective augmentative biological control options are available (Drummond & Groden, 2000). Moreover, pollination by bees is crucial for wild blueberry fruit set and the majority of growers utilize managed bees – honey bees, bumble bees or alfalfa leafcutting bees – during crop production. Thus, although producers remain heavily reliant on chemical insecticides to manage *I. argillacearia*, new products with demonstrated safety to growers, natural enemies and pollinators would be highly desireable.

Replacement of broad spectrum organophosphorous insecticides with reduced-risk pest management alternatives has been identified as a key research issue in wild blueberry production (Anonymous, 2005). In this study the potential of several reduced-risk insecticides for management of *I. argillacearia* was examined in the laboratory. Compounds representative of two insecticide classes were tested: flubendiamide (Belt 240 SC®, Bayer CropScience Canada Inc., Calgary, AB), a phthalic acid diamide; and spinetoram (Delegate WG®, Dow AgroSciences Canada Inc., Calgary, AB), a 'second generation' spinosyn insecticide. The primary action of flubendiamide is disruption of the calcium channel ryanodine receptors in lepidopteran insects (Kato *et al.*, 2009). This compound has little to no activity on insects in other orders (Hall, 2007).

It is anticipated that flubendiamide will soon be registered and readily adopted by blueberry growers for *I. argillacearia* management. Spinetoram was registered for use against *I. argillacearia* in wild blueberry in 2008. Spinetoram is formed through fermentation of the soil bacteria *Sacchoropolyspora spinosa* Mertz and Yao, and is highly specific to insects, particularly lepidopterans and dipterans (Thompson *et al.*, 2000; Kirst, 2010). As a comparison, the industry standard pyrethroid insecticide, deltamethrin (Decis 5 EC®, Bayer CropScience Canada Inc., Calgary, AB), was included in tests. Deltamethrin has been available for use in blueberry fields since 2002 and has been used to control *I. argillacearia* for the past several years.

2.2 Methods

2.2.1 Insects

Itame argillacearia larvae used in laboratory experiments were collected from fields in early June, intermittently over several days, using a 30 cm diameter sweep net. Larvae were placed in 6 L sealable plastic containers with blueberry stems during transport back to the laboratory. In the lab, larvae were maintained in 6 L plastic containers lined with paper towel at 4° C under a 0:24 light:dark (L:D) regime. Fresh blueberry foliage was replaced as needed. Larvae used in bioassays ranged from second to fourth instar and were assigned randomly to treatments in all laboratory experiments. Larvae were collected from blueberry fields near Collingwood Corner, Nova Scotia (45° 34.344’ N 063° 51.350’ W) and Tracadie, New Brunswick (47° 23.698' N 064° 58.450’ W); sites of major blueberry production and intense management in their respective

provinces. For the time-mortality bioassays, only larvae collected from the lowbush blueberry fields near Collingwood, Nova Scotia were used.

2.2.2 LC50 Determinations

Two biorational insecticides, flubendiamide and spinetoram, were compared with deltamethrin. Insecticides were suspended in deionized water with two to three drops of Tween-80 (Sigma-Aldrich Canada Ltd., Oakville, ON) to give stock solutions of 1000 ppm. Dilutions were subsequently made to give a range of concentrations that caused approximately 5-95% mortality. Water controls were used in all bioassays.

An ingestion-residual assay was used to assess toxicity. Untreated blueberry stems (10-15 cm) were collected in the field, placed in plastic bags and transported to the lab. Stems were immersed in the desired insecticide concentration for approximately 30 s and placed on wire racks until dry. The petiole of each stem was inserted into a floral water pick (Sproule Enterprises Inc., Mississauga, ON) containing deionized water to maintain freshness. The single water picks were then inserted upright into the bottom of 621 mL polystyrene cups (TRA Cash & Carry, Truro, NS). Five larvae were placed on each stem and each cup was covered with a 100 mm glass Petri dish. Insects were allowed to feed at 21° C and 24:0 (L:D). Mortality was recorded at 48 h after treatment. Larvae were considered dead if they were unresponsive to gentle probing with a dissecting needle. For each insecticide bioassay replicate there were five treatment concentrations (plus a control) x five larvae per cup x three to four cups per concentration. There were three replicates for flubendiamide and spinetoram and one replicate for deltamethrin. Bioassays were repeated at least once on different days

depending on availability of larvae. Concentration–mortality regression lines were generated for each insecticide by probit analysis (Finney, 1952) using the PROC PROBIT procedure in SAS 9.1.2 statistical software package (SAS, 2001). Concentrations lethal to 50% (LC50) of the sample population of *I. argillacearia* larvae, confidence limits, and slopes were determined. LC50 ratios (LC50 of Collingwood population/LC50 of Tracadie population) were calculated to determine differences in susceptibility between populations (Robertson, 2007). If ratio confidence intervals did not contain 1, then values are significantly different.

2.2.3 Time Mortality Bioassays

In the experiments described above, there appeared to be differences among insecticides in time taken to induce lethal effects in *I. argillacearia.* An experiment was conducted to quantify these differences. Solutions of flubendiamide and spinetoram were prepared in deionized water as previously described at the lowest recommended field concentration (LRFC), and 1/2 LRFC, which was 100 ppm and 50 ppm respectively for both flubendiamide and spinetoram. Deltamethrin was not used for this study since, even at very low concentrations, the product acted very quickly and caused 100% mortality of *I. argillacearia* within 24 hours of exposure. Field-collected blueberry stems were immersed in insecticide solution, air-dried and placed in cups with a floral pick filled with water, as previously described. Five larvae were added to each cup and there were three to four replicate cups per insecticide concentration. Insect mortality was recorded for up to 96 hours at eight hour intervals. After drying, stems were weighed before and after experiments. The amount of wet frass produced per five larvae was

calculated as the difference in weight between clean cups before the addition of larvae, and the weight of cups at the end of the experiment containing frass deposited by feeding larvae. Regression lines were generated using SigmaPlot (Systat, 2008) for each chemical to estimate percent mortality at selected doses at specific times. Frass and stem weight were analyzed using analysis of variance (PROC GLM) in SAS (SAS, 2001).

2.3 Results and Discussion:

2.3.1 LC50 Determination for *I. argillacearia*

Insects from Tracadie were found, through the use of LC50 ratios, to be less susceptible to spinetoram and deltamethrin than insects from Collingwood while both populations exhibited similar susceptibility to flubendiamide (Table 1). The two populations had a 16-fold difference in susceptibility to deltamethrin, a 4-fold difference in susceptibility to spinetoram, but no significant difference in susceptibility to flubendiamide. Susceptibility was highest to deltamethrin, followed by spinetoram and flubendiamide, respectively. However, inter-insecticide differences to the three insecticides varied between and within the populations. For example, the difference in susceptibility to deltamethrin and flubendiamide for the Tracadie population was only approximately 7-fold, whereas the Collingwood population displayed approximately a 57-fold difference in susceptibility to these compounds. Natural variation may be a factor, but it is more likely that differences between Tracadie and Collingwood in deltamethrin use patterns caused the large difference in LC50 values.

Table 1. Slope, LC50, confidence limits, *chi*-square values and LC50 ratios at 48 h after exposure of *I. argillacearia* larvae from Collingwood, NS and Tracadie, NB to spinetoram, flubendiamide and deltamethrin

Compound	Population	*n*	Slope ± SE	LC50 (ppm)	95% C.L.	χ^{2} [b]	LC50 ratio (95%C.I.)
spinetoram	Collingwood	275	1.32 ± 0.23	0.0462	0.015 - 0.103	6.4*	4.38 (1.91- 10.04)
spinetoram	Tracadie	219	0.78 ± 0.14	0.200	0.116 - 0.407	4.4	
flubendiamide	Collingwood	260	0.77 ± 0.13	0.230	0.130 - 0.391	1.3	2.11 (0.44 – 10.16)
flubendiamide	Tracadie	212	0.99 ± 0.15	0.477	0.305 - 0.795	2.6	
deltamethrin	Collingwood	143	0.89 ± 0.26	0.00412	$8.5x10^{-07}$ - 0.025	7.0*	16.18 (1.48 – 176.12)
deltamethrin	Tracadie[a]	100	0.68 ± 0.63	0.0664	-	28*	

[a] not able to determine 95% C.L.

[b] values followed by an (*) are significant

Leonard *et al.* (1988) showed that LD50 values for tobacco budworm varied between sites and noted that fields that had pyrethroids applied for pest control showed higher resistance than fields that had not. They also found that cross resistance among pyrethroids is high. It is therefore likely that the differences between populations in susceptibility to deltamethrin are symptomatic of mounting tolerance in Tracadie (e.g. due to more frequent applications to control higher pest pressure). Spinetoram has been registered only since 2008 and flubendiamide is not yet registered for use in blueberries. It is encouraging that the intra-site differences in susceptibility to spinetoram and flubendiamide are minimal compared to the large difference in seen deltamethrin susceptibility. This suggests that cross resistance between deltamethrin and spinetoram or flubendiamide is of low risk, and therefore the newer compounds will be of use in deltamethrin resistance management. This is not unexpected since each has a different mode of action than deltamethrin, making cross resistance with other insecticide classes unlikely (Nauen *et al.*, 2007). Saleem *et al* (2008) found that the LC50 was high for *Spodoptera litura* Fabricius larvae exposed to deltamethrin while Tohnishi *et al* (2005) found the LC50 quite low for *S. litura* exposed to flubendiamide. These studies were both done on insects selected for pyrethroid resistance as well as insects collected randomly from fields.

Slopes of the probit regression lines were shallow in general compared with other studies (Huang *et al.*, 2006; Yin *et al.*, 2008; Sial *et al.*, 2010). This is likely because a range of instars were used in tests rather than a single instar only. In general, earlier instars are more susceptible to insecticides than later instars and if a range of insect

stages are used in a bioassay, heterogeneity in response increases, thus lowering the slope (Robertson, 2007).

2.3.2 *Itame argillacearia* Time Mortality Bioassays

The results of the time mortality bioassay show that spinetoram and flubendiamide act at different rates and that rate depends on exposure concentration. Slopes, intercepts and their respective standard errors are given in Table 2. Mortality increased over time for all treatments and insects died faster at higher concentrations, but activity of flubendiamide was much slower than that of spinetoram, and 96 h mortality never exceeded 75% for the flubendiamide 50 ppm treatment (1/2 LRFC) (Fig. 2). The time taken to see greater than 90% mortality after exposure to spinetoram treated blueberry foliage is consistent with observations of two to four days after field applications or when used against the grasshopper, *Catantops axillaris* Thunburg (El-Gammal & Mohamed, 2008).

Tohnishi et al (2005) reported that it can take up to four days to see activity from flubendiamide, which is in agreement with the results of the present study. Slower activity of flubendiamide is not unexpected since it effectively stops the insect from feeding as it constricts skeletal muscle (Kato et al., 2009). This suggests that insects on flubendiamide treated foliage would eat less. However, no significant differences were found between treatments in frass production ($F = 1.76$; $df = 4, 14$; $P = 0.193$) or blueberry stem weight ($F = 1.42$; $df = 4, 14$; $P = 0.280$) (Table 3).

Table 2. R^2 values, intercepts, and slopes for linear regressions comparing time-activity of spinetoram and flubendiamide when exposed to *I. argillacearia* on blueberry foliage.

Compound (ppm)	R^2	**Intercept (± SE)**	**Slope (± SE)**
spinetoram 50	0.75	3.27 (10.01)	2.40 (0.430)
spinetoram 100	0.70	17.58 (8.88)	3.57 (0.574)
flubendiamide 50	0.71	-7.97 (3.85)	0.85 (0.068)
flubendiamide 100	0.76	1.64 (5.33)	1.03 (0.094)

Table 3. The differences in cup weight (frass) and blueberry stems weight following exposure of *I. argillacearia* larvae to insecticide treated blueberry stems for four days

Treatment	**difference in stem weight g (95 % C.I.)**	**difference in cup weight g (95 % C.I.)**
Control	0.500 (0.440)	0.150 (0.100)
flubendiamide 50 ppm	0.138 (0.180)	0.063 (0.072)
fluebendiamide 100 ppm	0.400 (0.357)	0.075 (0.061)
spinetoram 50 ppm	0.183 (0.072)	0.017 (0.036)
spinetoraml 100 ppm	0.017 (0.031)	0.033 (0.034)

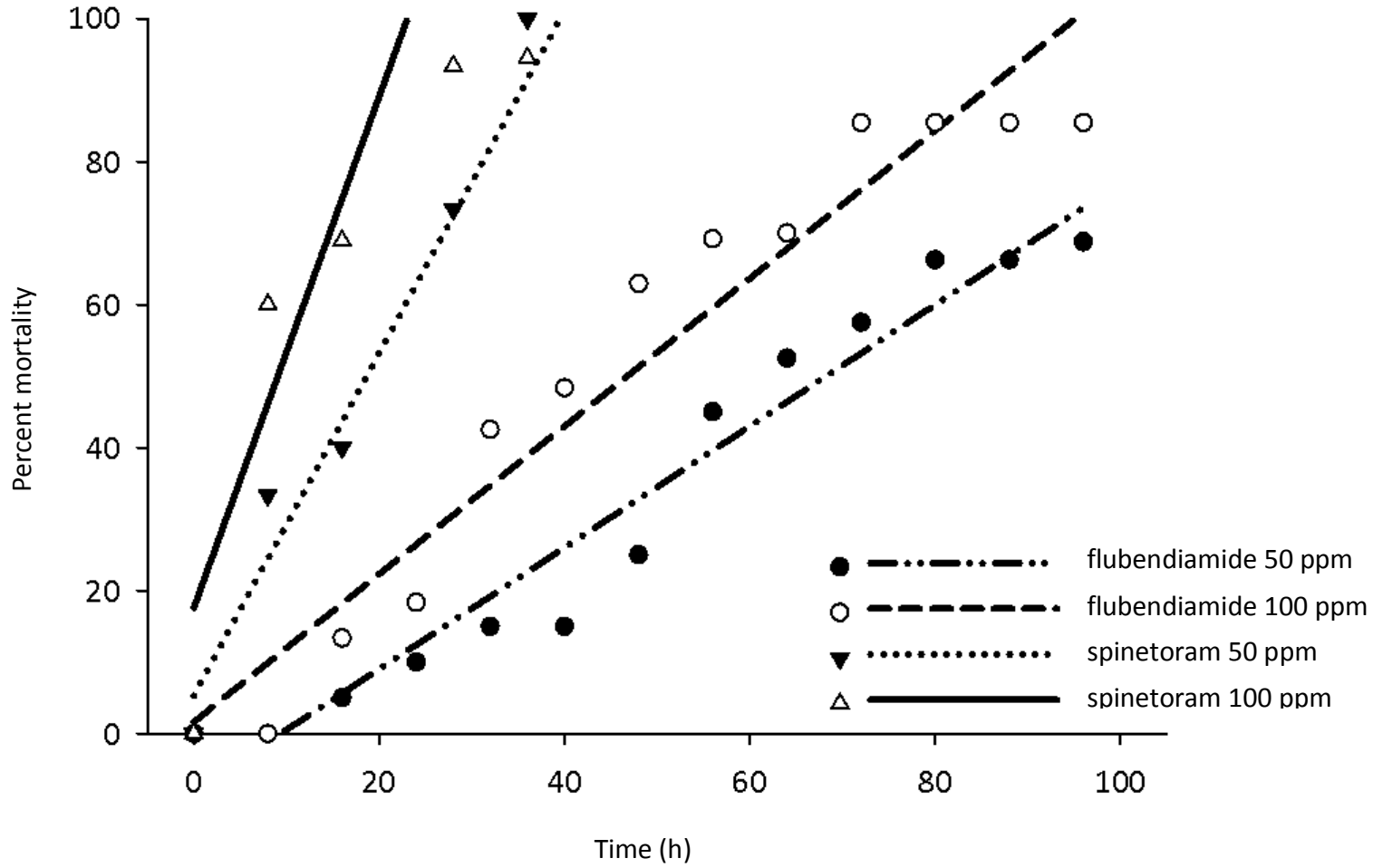

Figure 2. Regression lines illustrating *I. argillacearia* mortality over time when treated in the laboratory with flubendiamide or spinetoram at full (100 ppm) or 1/2 (50 ppm) LRFC.

Visually, there appeared to be less foliage in the control cups (100% of foliage was eaten in many control replicates) and the lack of significant effect was likely due to the fact that full stems were weighed (not foliage only) and that most weight was contained in stems.

Insects died quickly in the spinetoram treatments, with little to no foliage consumed at all. The spinosyns are nerve poisons, causing muscle paralysis and death, and are thought to act on an undefined subset of nicotinic acetylcholine receptors (Salgado, 1998; Perry *et al.*, 2007; Orr *et al.*, 2009), and can cause paralysis very quickly depending on the dose. In these experiments, 50 and 100 ppm caused mortality quickly within 48 hours (Fig. 2). It is therefore not unreasonable to expect that growers will see quick reductions in numbers of this pest in the field after application with spinetoram. Although, reduction in number of *I. argillacearia* will not be as dramatic when flubendiamide is used, it effectively stops the insect from feeding thus still providing economic control.

2.4 Conclusions:

These results provide estimates of baseline toxicity of flubendiamide, spinetoram and deltamethrin to *I. argillacearia* that will be useful in monitoring resistance to these compounds. The biorational compounds were highly active against this insect, although lethal concentrations are higher compared to deltamethrin, and flubendiamide is slower acting. Nonetheless, the potential added safety to applicators,

beneficial insects and the environment that flubendiamide and spinetoram provide, should make them a good fit for future *I. argillacearia* IPM programs.

CHAPTER THREE: SUSCEPTIBILITY OF *ITAME ARGILLACEARIA* TO INSECTICIDES TESTED IN THE FIELD

3.1 Introduction:

The blueberry spanworm, *Itame argillacearia* Packard is a major defoliator of wild blueberry, *Vaccinium angustifolium* Aiton., primarily feeding on leaves and developing flower buds, thus potentially lowering both berry number and weight. Wild blueberry producers generally control this pest using pyrethroid, organophosphorous (OP) or carbamate insecticides. Natural enemies do exist for *I. argillacearia*; but their numbers are thought to be too low to provide economic control of this pest (Drummond & Groden, 2000). Moreover, natural enemies and pollinators are affected by older broad spectrum insecticides such as the pyrethroid, deltamethrin, or the OP, phosmet, and thus biorational insecticides are desired. Fortunately, in recent years a number of selective, safer, biorational insecticides have become available, some of which have already been registered through Canada's Minor Use Pesticide registration program. Biorational insecticides are products that provide adequate insect control with an increased degree of safety to humans, and beneficial and non-target organisms (Djerassi *et al.*, 1974). Flubendiamide and spinetoram are new biorational insecticides with greater insect specificity and lower non-target impacts than many other currently available conventional insecticides (Dripps *et al.*, 2008; Sawaguchi *et al.*, 2009; Sial *et al.*, 2010). Spinosad, acetamiprid, *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin, and methoxyfenozide are also reduced-risk products that show promise for use in wild blueberry integrated pest management (IPM) programs.

In the present study, field trials were conducted to assess how well newer reduced-risk products controlled *I. argillacearia* in both crop and sprout fields compared to traditionally used insecticides.

3.2 Methods:

Small-plot field experiments were conducted at the following locations:

Mt. Stewart, Prince Edward Island (2008)	46° 37.95' N; 62° 90.06' W
Tracadie, New Brunswick (2009)	47° 22.47' N; 64°59.43' W
Debert, Nova Scotia (2009)	45° 25.20' N; 63° 28.20' W
Farmington, Nova Scotia (2010)	45° 35.046' N 063° 51.265' W
Kemptown, Nova Scotia (2010)	45° 30.008' N 063° 6.288' W

Plots (4 x 6 m) were arranged in a randomized complete block design with four replicates per treatment. Insecticides were applied at 200 - 230 L ha^{-1} using a hand-held R&D CO_2-propelled, 145-cm boom sprayer, with four flat spray XR80002VS TeeJet nozzles (48 cm spacing) operating at 241 kpa (Bellspray Inc., Opelousas, LA USA). Insecticides used were flubendiamide (Belt 240 SC®, Bayer CropScience Canada Inc., Calgary, AB), spinetoram (Delegate WG®, Dow AgroSciences Canada Inc. , Calgary, AB), spinosad (Entrust 80 W®, Dow AgroSciences Canada Inc. , Calgary, AB), methoxyfenozide (Intrepid 2F®, Dow AgroSciences Canada Inc. , Calgary, AB), acetamiprid (Assail®, Du Pont Canada Company, Mississauga, ON), *Beauveria bassiana* (Botanigard ES®, Koppert Canada Ltd., Scarborough, ON), deltamethrin (Decis® 5 EC, Bayer CropScience Inc., Calgary AB) and phosmet (Imidan 50 WP Instapak®, Gowan LLC. Yuma, AZ). The experiment at Mt. Stewart was done in the summer of 2008 from June 6 to June 25,

2008. The experiment in Tracadie was conducted from June 5 to July 8, 2009. All the experiments in 2010 were done between June 2, 2010 and June 29, 2010. All treatments are given in Table 4.

All fields were in the crop cycle of production with the exception of Tracadie, which was in a '2nd year crop' phase (sometimes done to synchronize production cycles of fields) and Farmington being in the sprout phase. Applications were made in early June and always under warm, sunny conditions, with a little or no wind. Larvae were generally second-to fourth instar and blueberry plants in the tight to loose cluster stage. Assessments in each plot were conducted in the mornings with a 30 cm diameter sweep net on various days up to 35 days after treatment (DAT). Sampling consisted of 10 sweeps per plot through the central area of each plot avoiding the plot boundaries. *Itame argillacearia* larvae were counted and then systematically distributed back into the same plot. In 2008, yield was assessed by collecting a sample of ripe berries from a 1 m^2 quadrat within each plot.

Normal distribution and equal variance assumptions were verified for the data. Data were analyzed by ANOVA ($\alpha = 0.05$). *Itame argillacearia* data from Mt. Stewart in 2008 did not meet normality and constant variance assumptions and were transformed by a $\log_{10}(x+1)$ transformation. In 2010, *I. argillacearia* data from Kemptown and Debert required a square root transformation as they did not meet normality and constant variance assumptions. Back-transformed means and 95% C.I. are reported for ease of reading. Means were separated by a Tukey's means separation test ($\alpha = 0.05$) and analyses were conducted with JMP 8.0.2 software (SAS, 2009).

Table 4. Product and rates for each experimental site in *I. argillacearia* insecticide field experiments.

Treatments for field experiments (g a.i. ha^{-1})					
Mt. Stewart site 1	**Mt. Stewart site 2**	**Tracadie**	**Kemptown**	**Debert**	**Farmington**
Control	Control	Control	Control	Control	Control
spinetoram (105)	spinetoram (105)	spinetoram (50)	spinetoram (50)	spinetoram (50)	spinetoram (25)
spinetoram (50)	spinetoram (50)	Botanigard (6.5)	spinetoram (25)	spinetoram (25)	acetamiprid (120)
methoxyfenozide (120)	methoxyfenozide (120)	Botanigard (3.9)	spinosad (105)	spinosad (105)	flubendiamide (35)
methoxyfenozide (240)	methoxyfenozide (240)	flubendiamide (70)	spinosad (66)	spinosad (52)	
spinosad (105)	spinosad (105)	flubendiamide (35)	flubendiamide (35)		
flubendiamide (140)	flubendiamide (140)	deltamethrin (6.25)	phosmet (1000)		
flubendiamide (70)	flubendiamide (70)				
phosmet (1000)	phosmet (1000)				

3.3 Results and Discussion:

Most of the treated plots had significantly fewer *I. argillacearia* larvae in the 2008 experiment at both site 1 ($F = 54.69$; $df = 8, 29$; $P < 0.001$), and site 2 ($F = 49.06$; $df = 8, 29$; $P < 0.001$) three days after treatment (Tables 5 and 6). This was encouraging since the pest infestation at the two Mt. Stewart sites were well above the recommended action threshold level of 12 larvae per 25 sweeps (or five larvae per 10 sweeps per plot) in crop fields (Crozier, 1995). For methoxyfenozide, there was some initial delay, evident in the data for 3 DAT, in insecticidal activity compared to other products. However, this was expected as methoxyfenozide is an ecdysone agonist, an insect growth regulator that interferes with the insect molting process and usually require several days to elicit observable effects (Smagghe *et al.*, 2003; Amiri-Besheli, 2010). All other treatments, at high and low rates, resulted in sharp decreases in *I. argillacearia* collections by 3 DAT, at levels comparable to the phosmet treatment. Moreover, a single application of each biorational product suppressed *I. argillacearia* populations in treatment plots for the duration of the experiment.

Table 5. Number of *I. argillacearia* larvae collected from experimental plots treated with different insecticides, Mt. Stewart, PEI, site 1, 2008.

	Mean (± 95% C.I.) number of *I. argillacearia* per 10 sweeps					
Treatment (g. a.i ha^{-1})	**0 DAT[1]**	**3 DAT**	**6 DAT**	**10 DAT**	**13 DAT**	**19 DAT**
Control	18.50 (5.10) a*	18.75 (10.63) a	14.75 (5.17) a	7.50 (2.11) a	4.75 (1.02) a	1.00 (0.43) a
spinetoram (50)	37.25 (9.58) a	1.00 (1.02) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b	0.25 (0.23) a
spinetoram (105)	30.50 (8.27) a	1.25 (0.97) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) a
methoxyfenozide (120)	22.75 (6.24) a	7.25 (4.31)b	0.75 (0.52) b	0.00 (0.00) b	0.25 (0.26) b	0.00 (0.00) a
methoxyfenozide (240)	24.50 (6.45) a	12.50 (6.83) ab	0.75 (0.52) b	0.25 (0.31) b	0.00 (0.00) b	0.00 (0.00) a
spinosad (105)	28.00 (7.61) a	0.75 (0.86) b	0.25 (0.40) b	0.25 (0.31) b	0.25 (0.21) b	0.00 (0.00) a
flubendiamide (70)	20.00 (5.47) a	5.25 (3.41) b	0.25 (0.40) b	0.25 (0.31) b	0.00 (0.0) b	0.00 (0.00) a
flubendiamide (140)	16.25 (4.34) a	2.50 (1.50) b	0.25 (0.40) b	0.25 (0.31) b	0.00 (0.00) b	0.00 (0.00) a
phosmet (1000)	28.25 (7.60) a	0.50 (0.72) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b	0.50 (0.33) a

*Means within columns followed by different letter are significantly different (Tukey multiple means comparison $\alpha = 0.05$)

[1] Pre-spray count; DAT = Days after treatment

Plots were treated directly after pre-spray count on June 6, 2008

Table 6. Number of *I. argillacearia* larvae collected from experimental plots treated with different insecticides, Mt. Stewart, PEI, site 2, 2008.

	Mean (± 95% C.I.) number of *I. argillacearia* per 10 sweeps					
Treatment (g. a.i ha^{-1})	**0 DAT[1]**	**3 DAT**	**6 DAT**	**10 DAT**	**13 DAT**	**19 DAT**
Control	24.25 (13.56) a*	15.75 (9.08) a	9.00 (1.80) a	7.25 (2.06) a	3.75 (0.53) a	2.25 (0.72) a
spinetoram (50)	29.50 (14.17) a	0.50 (0.72) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b
spinetoram (105)	22.75 (12.06) a	0.50 (0.72) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b
methoxyfenozide (120)	31.75 (16.14) a	7.00 (3.94) ab	0.25 (0.22) b	0.25 (0.32) b	0.00 (0.00) b	0.25 (0.28) b
methoxyfenozide (240)	21.75 (12.20) a	6.50 (3.76) ab	0.00 (0.00) b	0.25 (0.32) b	0.00 (0.00) b	0.00 (0.00) b
spinosad (105)	21.50 (11.22) a	0.50 (0.72) b	0.25 (0.22) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b
flubendiamide (70)	23.75 (12.30) a	3.50 (2.46) b	0.00 (0.00) b	0.25 (0.32) b	0.00 (0.00) b	0.25 (0.28) b
flubendiamide (140)	30.00 (12.30) a	3.25 (1.84) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b	0.25 (0.28) b
phosmet (1000)	19.25 (10.82) a	0.75 (0.86) b	0.00 (0.00) b	0.25 (0.32) b	0.00 (0.00) b	0.00 (0.00) b

*Means within columns followed by different letter are significantly different (Tukey multiple means comparison $\alpha = 0.05$)

[1] Pre-spray count; DAT = Days after treatment

Plots were treated directly after pre-spray count on June 6, 2008

Infestation levels at the experimental sites in Tracadie, NB in 2009 were well below those in 2008 at Mt. Stewart (Table 7). There was a post–application difference between the spinetoram, deltamethrin and flubendiamide treatments and the control ($F = 7.39$; $df = 6, 21$; $P < 0.001$), but numbers of *I. argillacearia* larvae in the control plots also decreased by large amounts soon after application of products. It was unclear why the rapid drop in the control plots occurred. Possibly, applications were made after the peak in the larval populations which was followed by an inevitable decline. In Mt. Stewart in 2008, although populations overall were higher, collections in control plots also decreased just after the start of the experiment.

The *B. bassiana* treatments did not provide any control of *I. argillacearia* at Tracadie in 2009 (Table 7). There may be several reasons for the lack of effect. For example, field applications targeting the European corn borer, *Ostrinia nubilalis* Hubner did not result in significant reductions of tunnels or numbers of corn borer (Lewis *et al.*, 1996), and results were variable when *B. bassiana* was field tested against the diamond back moth *Plutella xylostella* Linnaeus (Vandenberg *et al.*, 1998). The authors noted that efficacy was affected by larval instar. This entomopathogenic fungus has also demonstrated its effectiveness against a number of lepidopteran insect pests including *I. argillacearia* (Bing & Lewis, 1991; Drummond & Groden, 2000; Zurek & Keddie, 2000), but *B. bassiana* is not generally used for control of lepidopteran pests as results are inconsistent. *Beauveria bassiana* is effective against insects such as the tarnished plant bug and western flower thrip (Al-mazra'awi *et al.*, 2006).

Table 7. Number of *I. argillacearia* larvae collected from experimental plots treated with different insecticides, Tracadie, NB, 2009.

	Mean (± 95%C.I.) number of *I. argillacearia* per 10 sweeps				
Treatment (g a.i. ha^{-1})	**0 DAT[1]**	**4 DAT**	**10 DAT**	**13 DAT**	**20 DAT**
Control	6.25 (0.94) a*	1.50 (0.98) a	0.25 (0.49) a	1.25 (1.23) a	0.25 (0.49) a
flubendiamide (70)	4.75 (0.49) abc	0.00 (0.00) b	0.00 (0.00) a	0.00 (0.00) b	0.00 (0.00) b
flubendiamide (35)	4.25 (1.67) b	0.00 (0.00) b	0.00 (0.00) a	0.00 (0.00) b	0.00 (0.00) b
spinetoram (50)	5.00 (2.11) abc	0.00 (0.00) b	0.00 (0.00) a	0.00 (0.00) b	0.00 (0.00) b
B. bassiana (6.5)	5.00 (0.80) abc	1.25 (1.23) a	0.50 (0.89) a	1.25 (1.47) a	0.25 (0.49) a
B. bassiana (3.9)	5.75 (0.93) ab	1.00 (1.38) ab	1.75 (1.67) b	1.50 (1.27) a	0.00 (0.00) b
deltamethrin (6.25)	3.75 (1.23) c	0.00 (0.00) b	0.00 (0.00) a	0.00 (0.00) b	0.00 (0.00) b

*Means within columns followed by different letter are significantly different (Tukey multiple means comparison $\alpha = 0.05$)

[1] Pre-spray count; DAT = Days after treatment

Plots were treated directly after pre-spray count on June 5, 2009

Collins and Drummond (2001) found that *B. bassiana* was ineffective against *I. argillacearia* in Maine blueberry field trails. Second, all treatments in the present experiment were applied in the morning under warm sunny conditions. This was done to mimic a realistic application scenario by a grower. *Beauveria bassiana* is, however, susceptible to photodegradation (Gardner *et al.*, 1977) and conditions of low humidity are not conducive to optimal *B. bassiana* performance (Shipp *et al.*, 2003). Finally, in subsequent experiments in the field and lab with *I. argillacearia* and other insects, *B. bassiana* did not perform well[2] (C. Cutler unpublished data). This suggests that the product in general, or at least this particular strain/batch of *B. bassiana*, may be ineffective for control of *I. argillacearia*.

In Kemptown, NS, in 2010, flubendiamide, spinetoram and spinosad performed just as well as the industry standard, phosmet, and significantly reduced insect densities below levels found in the control plots ($F = 49.18$; $df = 4, 21$; $P = 0.001$) (Table 8). In Debert, NS, in 2010 there were some reductions following spinosad and spinetoram applications ($F = 1.67$; $df = 4, 15$; $P < 0.012$), but *I. argillacearia* densities were generally low in all treatments throughout the experiment (Table 9).

[2] Dr. F. A. Drummond. Professor, University of Maine, USA. Personal Communication. July 2009

Table 8. Number of *I. argillacearia* larvae collected from experimental plots treated with different insecticides, Kemptown, NS, 2010

	Mean[a] (± 95%C.I.) number of *I. argillacearia* per 10 sweeps				
Treatment (g a.i. ha^{-1})	**0 DAT[1]**	**2 DAT**	**6 DAT**	**14 DAT**	**17 DAT**
Control	3.25 (2.01) a*	2.25 (1.41) a	1.25 (0.61) a	0.75 (0.32) a	0.25 (0.09) a
flubendiamide (35)	2.25 (1.45) a	0.25 (0.24) b	0.25 (0.16) b	0.00 (0.00) b	0.00 (0.00) b
spinetoram (50)	1.75 (1.28) a	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b
spinetoram (25)	2.50 (1.73) a	0.75 (0.42) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b
spinosad (105)	3.25 (2.00) a	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b
spinosad (66)	3.25 (1.94) a	0.25 (0.24) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b
phosmet (1000)	3.50 (2.07) a	0.25 (0.24) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b

[a] Means were square root transformed before analysis. Back transformed means are presented to facilitate ease of reading

*Means within columns followed by different letter are significantly different (Tukey multiple means comparison $\alpha = 0.05$)

[1] Pre-spray count; DAT = Days after treatment

Plots were treated directly after pre-spray count on June 8, 2010

Table 9. Number of *I. argillacearia* larvae collected from experimental plots treated with different insecticides, Debert, NS, 2010

	Mean (± 95% C.I.) number of *I. argillacearia* per 10 sweeps					
Treatment (g a.i. ha^{-1})	**0 DAT[1]**	**2 DAT**	**7 DAT**	**12 DAT**	**14 DAT**	**16 DAT**
Control	1.75 (0.49) a*	1.00 (1.38) a	1.00 (1.38) a	0.50 (0.98) a	0.00 (0.00) a	0.50 (0.57) a
spinetoram (50)	3.25 (1.47) a	0.25 (0.49) ab	0.50 (0.56) ab	0.00 (0.00) a	1.00 (0.80) b	0.00 (0.00) a
spinetoram (25)	2.25 (0.94) a	0.00 (0.00) b	0.25 (0.49) ab	0.25 (0.49) a	0.00 (0.00) a	0.00 (0.00) a
spinosad (105)	2.00 (1.38) a	0.25 (0.49) ab	0.50 (0.57) ab	0.25 (0.49) a	0.00 (0.00) a	0.00 (0.00) a
spinosad (52)	3.00 (0.80) a	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) a

*Means within columns followed by different letter are significantly different (Tukey multiple means comparison $\alpha = 0.05$)

[1] Pre-spray count; DAT = Days after treatment

Plots were treated directly after pre-spray counts on June 2, 2010

Similarly, at the Farmington site, all treatments reduced the numbers of *I. argillacearia* when compared to the control (Table 10) (F = 7.43; df = 3, 14; P = 0.012), but, from 13 to 16 days after treatment, numbers of insects were low in all plots, although numbers in the control plots did increase slightly 20 days after treatment.

Results from the field experiments show that flubendiamide, spinetoram, acetamiprid, and methoxyfenozide work as well as phosmet or deltamethrin, the products generally used in *I. argillacearia* management. Flubendiamide has performed well against lepidopteran pests in other field trials at rates similar to those used in this study. Prasad et al (2010) found that flubendiamide rates of 24 g a.i. ha^{-1} gave high reduction in *Scirpophaga incertulas* Walker (yellow rice stem borer) infestations, while Kay and Brown (2009) used 72 g a.i. ha^{-1} to achieve economically significant reductions of *Sceliodes cordalis* DoubleDay. Flubendiamide is thought to affect all lepidopterans and thus differences in effective control among species should be minimal (Hirooka *et al.*, 2007). Likewise, the efficacy of spinosad, against *I. argillacearia* was comparable to that with other lepidopteran pests (Maxwell & Fadamiro, 2006). As far as I am aware, there have been no peer reviewed studies assessing the field efficacy of spinetoram to a lepidopteran insect pest. Acetamiprid effectively reduced larvae numbers, although it is generally used to control grubs and sucking pests (Elbert *et al.*, 2008). Yield, based on a sample from 1 m^2 from each plot, was recorded in 2008, but no significant treatment effects were found for site 1 (F = 1.16; df = 8, 27; P = 0.359) and 2 (F = 2.13; df = 8, 27; P = 0.068) (Table 11).

Table 10. Number of *I. argillacearia* larvae collected from experimental plots treated with different insecticides, Farmington, NS, 2010

	Mean (± 95% C.I.) number of *I. argillacearia* per 10 sweeps					
Treatment (g a.i. ha^{-1})	**0 DAT[1]**	**3 DAT**	**5 DAT**	**13 DAT**	**16 DAT**	**20 DAT**
Control	2.50 (3.31) a*	2.83 (5.59) a	0.50 (0.53) a	0.17 (0.53) a	0.50 (0.80) a	2.50 (2.41) a
flubendiamide (35)	0.00 (0.00) a	0.25 (0.49) b	0.00 (0.00) b	0.25 (0.65) a	0.00 (0.00) a	0.00 (0.00) a
acetamiprid (120)	2.75 (4.76) ab	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) a	0.00 (0.00) a	0.25 (2.95) a
spinetoram (25)	5.25 (4.76) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) a

*Means within columns followed by different letter are significantly different (Tukey multiple means comparison $\alpha = 0.05$)

[1] Pre-spray count; DAT = Days after treatment

Plots were sprayed directly after pre-spray counts on June 11, 2010

Table 11. Mean yield of wild blueberries from sites 1 and 2 at Mt. Stewart PEI from 2008

	Mean yield g m^{-2} (± 95% C.I.) of wild blueberries per plot	
Treatment (g a.i. ha^{-1})	**Site 1 Mt. Stewart PEI**	**Site 2 Mt. Stewart PEI**
Control	449 (127) a	333 (162) a
flubendiamide (140)	453 (247) a	621 (112) a
flubendiamide (70)	259 (117) a	394 (70) a
spinetoram (105)	470 (143) a	418 (91) a
spinetoram (50)	336 (205) a	505 (203) a
spinosad (105)	386 (135) a	480 (137) a
methoxyfenozide (120)	395 (154) a	683 (104) a
methoxyfenozide (240)	383 (128) a	451 (56) a
Phosmet (1000)	440 (112) a	356 (95) a

*Means within columns followed by different letter are significantly different (Tukey multiple means comparison $\alpha = 0.05$)

At site 1, yield ranged from 260 g m^{-2} to 450 g m^{-2} whereas at site 2, the range was from 330 g m^{-2} to 680 g m^{-2}. Even with such high infestations in 2008, no significant difference in yield was observed. Since subsequent years had very low infestations, impacts on yield would be less pronounced and they were not assessed. However, if there were more samples collected per plot, an effect may have been observed.

3.4 Conclusion:

These field experiment results show that there are several potential biorational insecticide alternatives for *I. argillacearia* management in wild blueberries. The efficacies of flubendiamide, spinetoram, spinosad, methoxyfenozide and acetamiprid were comparable to those of phosmet and deltamethrin, older chemistries that are used for *I. argillacearia* control. The higher application rates of the biorationals were much lower than that of the organphosphorous insecticide phosmet – up to 20-fold lower in some cases – meaning that in addition to reducing hazard to humans and non-target organisms, adoption of these biorational compounds will result in smaller amounts of insectcide entering the environment. However the rates for biorationals were much higher than for deltamethrin. Their real advantage in relation to deltamethrin is therefore not the lower rate of active ingredient but their much narrower spectrum of activity and possibly a shorter residual life. Moreover, most products worked quickly and a single application proved effective enough to suppress *I. argillacearia* populations over several weeks.

CHAPTER FOUR: SUSCEPTIBILITY OF *BOMBUS IMPATIENS* TO PESTICIDES USED IN WILD BLUEBERRY AGROECOSYSTEMS

4.1 Introduction:

Bombus impatiens Cresson is a commercially available bumble bee that is native to eastern North America (Finnamore & Neary, 1978). It is a very effective pollinator of many plants native to its region, including *Vaccinium angustifolium* Aiton. (wild blueberry) (Heinrich, 1979). Using "buzz pollination" (Buchmann, 1983), *B. impatiens* pollinate blueberry flowers more efficiently than almost any other wild bee, and may transfer four-fold more blueberry pollen per visit than honey bees (Javorek *et al.*, 2002). They are also prefer to visit blueberry flowers over other flowers in the field (Whidden, 1996)(Stubbs & Drummond, 1997). During commercial wild blueberry production, many insects begin to hatch and feed around the same time that blueberry flowers bloom. Thus, when insecticides are applied for control, bees that are pollinating blueberry flowers are often at high risk of coming into contact with pesticides. Since bees are also in the field well after bloom because there are many other forage options, pesticides applied to control later emerging pests may also have an impact on bees. Producers always attempt to apply pesticides when bees are less likely to be foraging (e.g. early in the morning or in the evening) but this is not always possible. Therefore, growers desire reduced risk insecticides and bio-pesticides that offer good management of insect pests, with minimal risk to pollinators.

Several reduced risk pest management products and biopesticides are currently being considered for pest management in wild blueberry. *Bacillus subtilis* (Ehrenberg)

Cohn is a gram positive soil dwelling bacteria that has fungicidal activity (Nakano & Zuber, 1998; Mantecon, 2008). Several strains of this microbe have been formulated into sprays that are used to control many fungal pathogens in agroecosystems (Gueldner *et al.*, 1988; Elizabeth & Jo, 1999). In blueberries, it is used for control of *Botrytis spp* fungi, which causes botrytis floral blight (Bernard *et al.*, 1998; Elad & Stewart, 2004).

Spirotetramat is a novel chemical developed by Bayer CropScience mainly for control of sucking insect pests. It inhibits lipid synthesis; affecting juvenile stages and adult fecundity. It has been shown to be very effective against a wide variety of aphids, thrips, scales, mealy bugs and whitefly species (Bruck *et al.*, 2009). However, spirotetramat has also demonstrated very good potential for control of blueberry maggot, *Rhagoletis* mendax (Curran), and registration in blueberries is possible in the near future[3]. Tests on beneficial arthropods show that spirotetramat has low toxicity to ladybird beetles, predatory mites, and spiders (Maus, 2008). However, recent controversy over the toxicity of spirotetramat to honey bees (*Apis mellifera* Linnaeus) has resulted in the formulated product being deregistered in the US (Erickson, 2010). Thus, further evaluation of the toxicity of this compound to bees is required.

Beauveria bassiana (Bals.-Criv.) Vuill. GHA is a naturally occurring fungal pathogen that attaches to the cuticle of an insect through hydrophobic interactions of a hydrophobin protein on the conidia and infects through the cuticle (Lefebvre, 1934). It is registered as a foliar spray for blueberries in the US and tests against blueberry pests

[3] Dr. Chris Cutler, Assistant Professor, NSAC. Personal communication. October 22 2010

in Canada have been done. *Beauveria bassiana* mildly affects *A. mellifera,* but seems to have a stronger effect on *Bombus terrestris* Linnaeus (Alves *et al.*, 1996; Hokkanen *et al.*, 2003). It is used for control in greenhouse agroecosystems and the dry formulation at field rates for control of thrips and whiteflies has been shown to be safe enough to *B. impatiens* in this system, enabling *B. impatiens* to be used as a vector for this biopesticide (Kapongo, Shipp, Kevan, & Sutton, 2008).

With bee health and declines now of increasing global concern, research on acute and sub-lethal toxicity of pesticides to bees is critical to ensure sustainable agroecosystems. Although all products registered in North America for pest management purposes must be tested against *A. mellifera,* toxicity tests on other bee species are not required. Thus, relatively little information is known of the effects of pesticides on *B. impatiens*. This information is important not only to assess risk to native populations of *B. impatiens*, but also for risk to managed colonies that are increasingly used for the pollination of a variety of crops. Thus the objectives of this study were to investigate the long-term toxicity of *B. bassiana*, *B. subtilis* and spirotetramat to *B. impatiens* after a one time topical exposure and to chronic oral exposure over 30 days.

4.2 Methods:

4.2.1 *Bombus impatiens*:

Bombus impatiens colonies were donated from Koppert Biological Systems (Scarborough, ON). Four Class A hives were received, two for the contact exposure

experiments and two for the oral exposure experiments. Each hive came with one fully developed queen and several developing queens plus 50-70 workers. Colonies eventually yielded about 300 workers with 50-70 workers emerging weekly.

Each colony was provided protein and sugar supplements from Koppert. Pollen (Cosmon and Whidden Honey, Greenwich, NS) was ground into a coarse powder and 10 mL, measured with a graduated cylinder, was placed in a small 28/35 mm hexagonal disposable polystyrene weigh dish (Fisher Scientific Company, Ottawa, ON). This was provided daily as an additional protein source and to accustom the bees to pollen feeding for subsequent toxicity bioassays. Bees were kept at 25° C in total darkness to mimic natural light condition within the hive.

4.2.2 Microcolonies:

Experimental microcolonies were set up in 461 mL plastic cups (TRA Cash & Carry Inc., Truro, NS). The bottom of each cup was cut out and covered with craft netting (6.35 mm mesh) secured with a rubber band. This first container was placed in a second 473 mL polystyrene container (TRA Cash & Carry Inc., Truro, NS) which contained a floral pic (Sproule Enterprises Ltd., Mississauga, ON) or glass vial feeder stuffed with cotton wool or wicks soaked with a 60:40 honey:water solution (honey provided by Cosmon and Whidden Honey, Greenwich, NS; or Kittleson Farms, Debert, NS) (Fig. 3). The feeder was placed under the craft netting giving bees easy access to the honey solution. Honey solutions and feeders were replaced twice per week. Each colony was given a 2 g pollen ball in a wax-coated polystyrene weighing dish during the first week of the experiment, and a 1 g pollen ball twice a week every week thereafter.

Figure 3. *Bombus impatiens* micro-colonies

Pollen balls were prepared by creating a paste of 5 parts pollen, 1 part honey and 1 part water. The paste was measured and rolled into 2 g and 1 g balls. The measured balls were dipped in melted wax until coated and left to cool and harden. Bee fecal matter dropped through the craft netting into the polystyrene containers and containers were changed twice a week. Bees were monitored and data was collected every 24 hours.

4.2.3 Direct contact experiments:

The biorationals used in this study were *B. subtilis* (Serenade Max® AgraQuest Inc. Davis CA), *B. bassiana* (Botanigard ES®, Koppert Canada Ltd., Scarborough, ON), and spirotetramat (Movento® Bayer CropScience Canada Inc., Calgary, AB). Bees were treated with the maximum recommended field concentration (MRFC), twice the MRFC, 1/10, 1/5 and 1/2 times the MRFC for each product. The corresponding quantities of these treatments are given in Table 12. Controls consisted of treatment with water only.

Four to seven day old bees were randomly drawn from the large hives and placed in mason jars with ventilated lids with ten to twelve bees per jar and a total of seven jars. Three bees were randomly selected out of any three jars and assigned to a treatment. All three bees were treated topically on the dorsal surface of their thorax before being placed in a microcolony. This was a completely randomized design. Bees were treated with 50 µL of insecticide using the PAX 100-3 automatic micro-dispensing system (Burkard Scientific, Uxbridge, UK). There were four replicate microcolonies per concentration of each biorational.

Table 12. Rates of treatments expressed as fraction of the field rate used for contact and ingestion exposure routes.

	Maximum recommended field rate concentrations (MRFC) for treatments		
	B. subtilis **(CFU[a] L^{-1})**	***B. bassiana*** **(CFU L^{-1})**	**spirotetramat (g A.I. L^{-1})**
1/10 MRFC	3.29×10^{12}	2.09×10^{09}	0.078
1/5 MRFC	6.57×10^{12}	4.20×10^{09}	0.156
1/2 MRFC[b]	1.64×10^{13}	1.05×10^{10}	0.390
MRFC[b]	3.83×10^{13}	2.09×10^{10}	0.780
2 MRFC[b]	6.57×10^{13}	4.18×10^{10}	1.560

[b] Only these concentrations were used for the ingestion exposure routes and were mixed with a 60:40 honey water solution instead of plain deionized water. Fresh solutions for these treatments were made every day that feeders were changed.

[a]CFU = colony forming units

All five concentrations for all four replicates were applied on the same date with each chemical being tested separately on a different week.Microcolonies were held in a walk-in incubator at 25° C under total darkness and 60% relative humidity.

Bees were checked once a day for 60 days. For each microcolony, data were collected for worker bee mortality, drone production, number of days to oviposition, and number of days to drone emergence. Previous studies have indicated that drone production, days to oviposition, days to drone emergence and larvae ejection can indicate toxic effects on colonies (Gradish *et al.*, 2009; Mommaerts *et al.*, 2009). Since the large hives only produced between around 50 to 70 workers weekly, experiments were staggered one week apart with the *B. subtilis* topical experiment being done first, followed by *B. bassiana* topical exposure, then spirotetramat topical exposure and all bees coming from the same two hives.

Worker mortality data could not be normalized and therefore was analyzed using a Kruskal-Wallis (non-parametric rank score) test since data did not fit normality and constant variance assumptions. Oviposition data was analyzed using analysis of variance (ANOVA). Data on the drones produced were analyzed using a linear mixed model (repeated measures analysis) with an auto-regressive covariance structure. Least-squares means from the linear mixed model were separated using a Tukey-Kramer test at $\alpha = 0.05$. Means were also separated by a Tukey-Kramer test at $\alpha = 0.05$ for oviposition and drone emergence. The Kruskal-Wallis test was done using Minitab 15 (Minitab, 2007). All other analyses were conducted using SAS 9.1.2 (SAS, 2001). Days to

oviposition for *B. bassiana* were square root transformed to fit normality and back-transformed means and confidence intervals are presented to facilitate ease of reading.

4.2.4 Oral Toxicity Experiment

Experiments were conducted that exposed bees orally to pesticides via pesticide-honey-water solution spiked with *B. subtilis*, *B. bassiana*, or spirotetramat. Treatments consisted of the maximum recommended field concentration (MRFC), twice the MRFC and one half times the MRFC prepared as mentioned previously but with a 60:40 honey:water solution instead of just water (Table 12). Bees were selected and randomized among treatments as mentioned previously. Bees in control microcolonies were given honey water only. Treatments were made fresh every time feeders were changed. There were five replicate microcolonies in each experiment. Bees were only exposed to treated honey water for 30 days and untreated honey water for the remaining 30 days. Like the topical exposure experiments, experiments were staggered one week apart with the *B. subtilis* experiment first, followed by *B. bassiana* exposure, then spirotetramat exposure and all bees coming from the same pair of hives. Two fresh hives were used for the oral exposure experiments.

Data collection, endpoints measured and statistical analysis were conducted as in the direct exposure treatment except that larval ejection data was also collected. Larval ejection data was analyzed with ANOVA and means separation was done according to Tukey's means separation tests. Data was analyzed using SAS 9.1.2 (SAS, 2001).

4.3 Results and Discussion:

4.3.1 Bumble Bee Contact Toxicity

There were no significant differences in *B. impatiens* worker survival after 60 days as a result of topical pesticide exposure to either *B. subtilis* (H = 6.48; df = 5; P = 0.262), *B. bassiana* (H = 0.85; df = 5; P = 0.974), or spirotetramat (H = 4.81; df = 5; P = 0.440) (Table 13). For *B. subtilis*, this was not unexpected as dry *B. subtilis* spores and dry formulated *B. subtilis* were not found to significantly infect honey bees through dermal contact (Prier *et al.*, 2001; Dedej *et al.*, 2004). Prier *et al* (2001) proposed that as bees enter the hive, they clean off the spores and that hive propolis (plant resin collected and mixed with wax by bees used for hive sterilization) has antifungal properties that arrest *B. subtilis* infections. Porrini *et al* (2010) did not find any acute toxicity of either surfactins or bacteriosins produced by *B. subtilis* to honey bees. However, *B. subtilis* did cause high mortality in *B. terrestris* through topical application (Mommaerts *et al.*, 2009). These conflicting results are possibly due to differences in susceptibility between species (Hokkanen *et al.*, 2003) but this seems an unlikely explanation for such a striking difference in results given that both bee species are in the same genus. Differences could also be due to differences in the formulation of *B. subtilis* used or other subtle differences in experimental conditions, such as nest size, number of workers originally treated and type of carbon source (e.g. sugar water vs honey).

Table 13. *B. impations* worker survival 60 days post exposure to topical treatments of *B. subtilis, B. bassiana* and spirotetramat

	Median number of worker bees[a] (average rank, IQR[b])		
Treatment	***B. subtilis***	***B. bassiana***	**Spirotetramat**
Control	2.5 (13.0, 2.50)	3.0 (13.1, 0.75)	3.0 (13.3, 1.50)
1/10 MRFC	3.0 (18.0, 0.75)	3.0 (12.3, 2.25)	2.5 (11.5, 1.00)
1/5 MRFC	0.5 (6.3, 1.75)	2.5 (10.3, 1.00)	2.0 (8.8, 0.75)
1/2 MRFC	2.0 (11.2, 2.25)	3.0 (13.1, 0.75)	2.5 (10.3, 2.50)
MRFC	1.5 (11.5, 1.75)	3.0 (13.1, 0.75)	3.0 (17.0, 0.00)
2 MRFC	2.5 (13.8, 2.5)	3.0 (13.1, 0.75)	3.0 (14.3, 0.75)

[a] Bees were monitored through 60 days with data recorded daily and number of workers counted at end of experiment. Data was ranked among all treatment levels and average ranks were calculated within treatment level.

[b] IQR = interquartile range for median value

Beauveria bassiana, in its dry formulation and at recommended rates for control of western flower thrip (*Frankliniella occidentalis* Pergande) and green peach aphid (*Myzus persicae* Sulzer), is generally not harmful to *B. impatiens*, thus making the bumble bee a possible vector for dry *B. bassiana* product (Al-mazra'awi *et al.*, 2006). Further studies investigated rates of *B. bassiana* in a dry formulation that optimally control pest insects but have low impact on *B. impatiens* (Kapongo, Shipp, Kevan, & Broadbent, 2008). However, it was recently found that the liquid formulated *B. bassiana* (Botanigard ES®) was toxic to *B. terrestris* when applied topically at the maximum recommended field rate (Mommaerts *et al.*, 2009). This difference may be due to differences in species susceptibility to this formulation (Hokkanen *et al.*, 2003) but again, given that the experimental results are so highly different and that these bees are in the same genus, this seems unlikely. The ability of *B. bassiana* to infect *B. impatiens* can also depend on temperatures within the hive that may inhibit growth of conidia (Goettel *et al.*, 1990). Thoracic temperature in honey bees and bumble bees is linearly related to sugar concentration in the diet (Nieh *et al.*, 2006). At a sucrose concentration of 2.5 mol L^{-1} and an ambient temperature of 25° C, thoracic temperatures in *Bombus wilmattae* Cockerrel, ranged from 28° C to 42° C (Nieh *et al.*, 2006). The combined concentration of fructose, glucose, sucrose and maltose in feeders for topical exposure to *B. bassiana* in the present study was at least 3.8 mol L^{-1}, and at 25° C, one might expect that the thoracic temperatures of *B. impatiens* used in this study to be consistently at the higher end of the temperature range mentioned for *B. wilmattae*. The optimal growing temperatures for *B. bassiana* can be as low as 20° C

or as high as 30° C and is greatly dependent on the fungal isolate (Fargues *et al.*, 1997), thus since the thoracic temperatures of the bumble bees in this study were most likely higher than 30° C, we may not see significant germination of *B. bassiana* and infection of *B. impatiens* (Kevan *et al.*, 2003). In contrast, Al-mazra'awi *et al* (2006) did see detrimental effects on *B. impatiens* when a surfactant (Tween-80, Sigma-Aldrich Canada Ltd., Oakville, ON) was added to the treatments. Thus, differences in formulation and possibly product batch may affect pathogenicity to bumble bees.

There was no mortality as a result of topical exposure to spirotetramat (Table 14). Since spirotetramat works best through ingestion, topical application was not expected to result in mortality. The contact LD50 for honey bees is above 100.0 µg a.i. bee^{-1} for the active ingredient, and 162.0 µg a.i. bee^{-1} for the OD 150 formulation (Maus, 2008). In this study, at the MRFC, we applied 0.780 g A.I. L^{-1}, which was equivalent to 3.9×10^{-5} µg a.i. bee^{-1}. This was significantly less than the amount tested by Maus (2008).

For *B. subtilis*, the average number of drones produced was significantly higher than the control for 1/5 and 1/2 MRFC ($F = 3.27$; $df = 5, 18$; $P = 0.028$) (Table 14). At 1/2 the MRFC of *B. subtilis*, we see a 36% increase in drone production over the controls. This type of increase may be indicative of a hormetic threshold model for dose response as seen with other organisms (Calabrese *et al.*, 2008; Cutler *et al.*, 2009; Guedes *et al.*, 2010). In the hormetic dose-response model, organism's exhibit modest stimulation at the low dose of a stressing agent that at higher doses would cause adverse effects (Calabrese & Baldwin, 2003).

Table 14. Least-squares means of endpoints of *B. impatiens* microcolonies 60 days after a one time topical treatment of *B. subtilis, B. bassiana*, and spirotetramat at different proportions of the maximum recommended field concentrations (MRFC) to worker bees.

		Means[a] (± 95% C.I.)		
Treatment	**Treatment level**	**Drones produced[b]**	**Days to oviposition**	**Days to drone emergence**
B. subtilis	control	11.50 (3.87) a*	n/a[d]	27.25 (2.22) a
	1/10 MRFC	13.00 (4.06) ab	n/a	29.00 (1.82) a
	1/5 MRFC	16.75 (4.64) bc	n/a	27.50 (1.29) a
	1/2 MRFC	18.00 (4.85) c	n/a	28.50 (2.64) a
	MRFC	10.25 (3.61) a	n/a	30.00 (4.08) a
	2 MRFC	13.50 (4.19) ab	n/a	30.00 (2.45) a
B. bassiana[c]	control	5.50 (1.00) a	6.04 (3.16) a*	39.50 (2.33) a
	1/10 MRFC	5.00 (1.00) a	6.00 (0.00) a	38.67 (4.57) a
	1/5 MRFC	6.25 (1.00) a	5.76 (2.32) a	36.50 (1.62) a
	1/2 MRFC	6.25(1.00) a	6.12 (4.24) a	44.00 (10.21) a
	MRFC	5.25 (1.00) a	4.74 (3.62) a	33.67 (2.85) a
	2 MRFC	5.25 (1.00) a	5.24 (4.14) a	37.75 (2.58) a
spirotetramat	control	5.54 (3.11) ab	4.75 (1.23) a	31.75 (2.02) a
	1/10 MRFC	6.27 (3.11) ab	3.75 (1.67) a	31.75 (3.61) a
	1/5 MRFC	6.62 (3.11) bc	3.25 (0.94) a	33.50 (3.71) a
	1/2 MRFC	7.17 (3.11) bc	3.00 (1.39) a	31.75(0.94) a
	MRFC	2.84 (3.11) a	3.50 (0.56) a	34.75 (5.44) a
	2 MRFC	3.79 (3.11) ab	3.75 (1.67) a	34.00 (2.89) a

[a] Bees were monitored over 60 days with data recorded daily
[b] Number of drones counted at end of experiment
[c]Dates to oviposition for *B. bassiana* were square root transformed. Back-transformed means and confidence intervals are presented to facilitate ease of reading
[d] Data for days to oviposition for *B. subtilis* was not recorded
*Least squares means for each exposure route within treatments followed by different letter are significantly different (Tukey multiple means comparison $\alpha = 0.05$)

Mommaerts *et al* (2009) found significantly lower drone production when compared to controls due to high mortality of workers after topical exposure to *B. subtilis*. However, as treatment concentration decreased, drone production increased since more workers were alive but the number of drones produced did not surpass levels seen in control microcolonies at any treatment such as in present study. Low dose stimulation of reproduction has been seen for other insects (Kuenen, 1958; Gordon & McEwen, 1984; Lowery & Sears, 1986), however, studies examining hormesis in insects generally focus on fecundity (Calabrese & Baldwin, 1999; Cutler *et al.*, 2009). This study found an increase in production of drones within one generation. This is the first study to demonstrate an increase in reproduction in *B. impatiens* when exposed to *B. subtilis*. Although drone production was affected, there were no significant effects on number of days to drone emergence ($F = 0.86$; $df = 5, 18$; $P = 0\ .529$) (Table 14).

For *B. bassiana*, there were no significant differences in drone production as a result of treatment ($F = 0.27$; $df = 5, 18$; $P = 0.922$) (Table 14). For spirotetramat there was a significant difference between the 1/2 and 1/5 MRFC, and the full MRFC but no significant difference in drone production between 1/2 MRFC and 1/5 MRFC, and the controls ($F = 1.39$, $df = 5, 18$; $P = 0.253$) (Table 14). There was no significant difference in days to oviposition for *B. bassiana* ($F = 0.94$; $df = 5, 18$; $P = 0.481$) or days to drone emergence ($F = 1.65$; $df = 5, 18$; $P = 0.204$) (Table 14). There was also no difference in days to oviposition ($F = 0.82$; $df = 5, 18$; $P = 0.548$) and days to drone emergence ($F = 0.59$; $df = 5, 18$; $P = 0.706$) for spirotetramat (Table 14). Mommaerts *et al* (2009) found that topical application of formulated *B. bassiana* at the recommended field rate

significantly reduced drone production when compared to the controls. This contrast with results in Table 14 may have been due to other factors such as number of insects treated, colony design, and temperature and humidity under which experiments were conducted. Since *B. bassiana* did not cause significant worker mortality in the present study, it is not unexpected that the colonies will produce drones since workers are alive to care for the brood. Conversely in the Mommaerts *et al* (2009) study, the low drone production was likely the result of low worker survival. Since spirotetramat is most effective through ingestion (Bruck *et al.*, 2009), it is not unexpected to see no significant differences to the control in days to oviposition, and days to first drone emergence.

For spirotetramat, although there was no significant effect of treatment on drone production, there was a significant treatment x time interaction effect (F = 1.25, df = 165, 592; P = 0.030) which means there were differences among treatments in the rate of drone production (Fig. 4). As drones first began to be produced, the control and the 1/10, 1/5 and 1/2 the MRFC treatments experienced rapid increase in drones. The high rate of production ended earlier for the control microcolonies than the 1/5 and 1/2 MRFC treatments. As spirotetramat does affect adult fecundity of honey bees (Maus, 2008), it is not unreasonable to expect that it may have an effect on drone production in *B. impatiens*.

The cumulative number of drones in controls did decrease from the *B. subtilis* exposure to the *B. bassiana* and spirotetramat exposure. This may be due to the first set of workers being more active or fertile than workers emerging later.

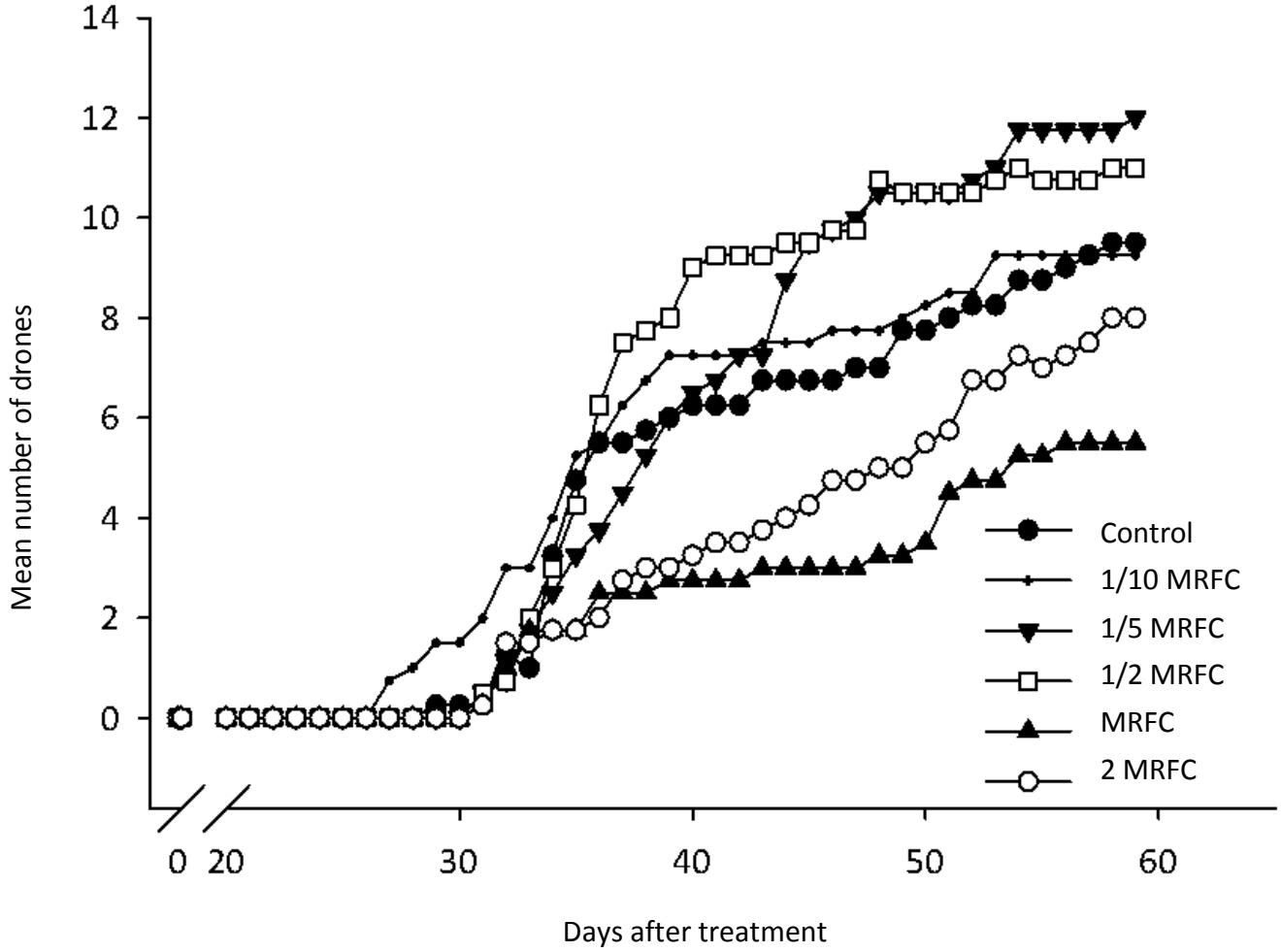

Figure 4. Drone production curves after a one time topical exposure of adult *B. impatiens* workers to spirotetramat

4.3.2 Bumble Bee Oral Toxicity

There were no significant differences in survival of worker *B. impatiens* after feeding on diluted honey treated with *B. subtilis* (H = 2.30; df = 3; P = 0.513) and *B. bassiana* (H = 4.93; df = 3; P = 0.177) (Table 15). However, there was high mortality after 10 days in microcolonies where worker bees were fed spirotetramat treated diluted honey when compared with control bees (H = 12.67; df = 2; P = 0.002) (Table 15). Oral exposure of spirotetramat to honey bees at the field rate concentration in a spiked sucrose solution did not cause significant mortality (Maus, 2008), but honey bee brood in hives has been shown to be negatively affected by oral over-exposure to field rates of spirotetramat via a contaminated carbohydrate source (Maus, 2008). Mommaerts *et al* (2009) found that *B. subtilis* oral treatments did not have a detrimental effect on survival of *B. terrestris* but oral treatments with *B. bassiana* did have a significant effect. The difference in results of bee survival for *B. subtilis* oral exposure may be due to bee species, or type of carbon source (e.g. sugar water or honey) used for mixing oral treatments. Previous studies have found *B. subtilis* to not adversely affect *B. impatiens* when applied as a dry powder or formulation (Dedej *et al.*, 2004; Ngugi *et al.*, 2005). Similarly, few other studies have looked into oral treatment of *B. bassiana* on bumble bees. The results presented here regarding bee survival agree with the results by Mommaerts *et al* (2009) where bees were orally exposed to *B. bassiana*.

Table 15. Worker *B. impatiens* survival 60 days post exposure to oral treatments of *B. subtilis, B. bassiana* and spirotetramat via treated honey water

	Median[a] (average rank, IQR[b])		
Treatment	***B. subtilis***	***B. bassiana***	**spirotetramat**
Control	3.0 (14.9, 0.50)	3.0 (12.0, 0.50)	3.0 (13.0, 0.00)
1/2 MRFC	2.0 (7.5, 1.00)	3.0 (8.0, 2.50)	0.0 (6.0, 0.50)
MRFC	2.0 (10.4, 1.00)	3.0 (10.0, 2.50)	0.0 (5.0, 0.00)
2 MRFC	2.0 (9.2, 0.50)	3.0 (12.0, 1.00)	

[a] Bees were monitored over 60 days with data recorded daily and number of workers counted at end of experiment. Data was ranked among all treatment levels and average ranks were calculated within treatment level.
[b] IQR = interquartile range of median values

Although low worker mortality was observed in the present study when *B. impatiens* was fed honey water treated with *B. subtilis*, there was a clear dose-response relationship on drones produced (Table 16) ($F = 4.46$; $df = 3, 16$; $P = 0.018$) with the number of drones decreasing as dose increased. There was a significant interaction effect between treatment and time ($F = 1.49$; $df = 84, 448$; $P = 0.006$) with cumulative drone production increasing more slowly as dose was increased, which is seen in the difference in shape of the growth curves (Fig. 5). Bees from *B. subtilis* treated microcolonies also oviposited later than control colonies ($F = 3.31$; $df = 3, 16$; $P = 0.047$) (Table 16). There was no significant difference in number of larvae ejected from treated colonies and controls ($F = 0.76$; $df = 3, 16$; $P = 0.530$) (Table 16). Drones also began to emerge later in treatment microcolonies than in controls ($F = 16.35$; $df = 3, 16$; $P < 0.0001$) (Table 16). *Bacillus* spp. has been shown to survive in synthetic nectar (Pusey, 1999; Mommaerts *et al.*, 2009) and Mommaerts *et al* (2009) found that mixing a sugar solution with formulated *B. subtilis* at the highest recommended field rate concentration resulted in high worker *B. terrestris* mortality and no drone production. Differences in results presented in this paper and those by Mommaerts *et al* (2009) (e.g. reduced drone production vs high worker mortality) may have been due to differences in bee species or perhaps in formulation (Serenade Max® in this study vs Serenade® in the study by Mommaerts). Also, the fact that there was no drone production in the study by Mommaerts *et al* (2009) is most likely due to the fact that no workers survived to raise the drones.

Table 16. Least-squares means of measured endpoints following exposure of *B. impatiens* microcolonies after 60 days to oral treatment of *B. subtilis*, *B. bassiana*, and spirotetramat at different proportions of the maximum recommended field concentrations via treated honey water.

		Means[a] (± 95% C.I.) letter groupings			
Product	**Treatment level**	**Drone production[b]**	**Days to oviposition**	**Larval ejection**	**Days to drone emergence**
B. subtilis	control	6.60 (1.85) a*	6.20 (1.56) a*	5.00 (4.15) a*	33.80 (1.44) a
	1/2 MRFC	4.80 (1.85) b	8.00 (4.60) ab	2.00 (2.56) a	43.00 (6.20) b
	MRFC	2.20 (1.85) c	8.20 (4.57) ab	2.60 (2.74) a	44.00 (2.26) b
	2 MRFC	2.80 (1.85) c	14.80 (4.65) b	2.80 (1.8) a	55.25 (4.11) c
B. bassiana	control	4.61 (2.97) a	6.00 (0.62) a	3.80 (3.47) a	34.67 (1.73) a
	1/2 MRFC	7.82 (2.97) a	5.40 (0.78) a	3.20 (2.87) a	33.00 (1.38) a
	MRFC	5.44 (2.97) a	5.20 (1.57) a	0.60 (1.18) a	33.25 (2.31) a
	2 MRFC	6.43 (2.97) a	6.00 (0.88) a	1.80 (2.66) a	34.25 (4.48) a

[a] Bees were monitored over 60 days with data recorded daily

[b] Number of drones counted at end of experiment

*Least squares means for each exposure route within columns followed by different letter are significantly different (Tukey multiple means comparison $\alpha = 0.05$)

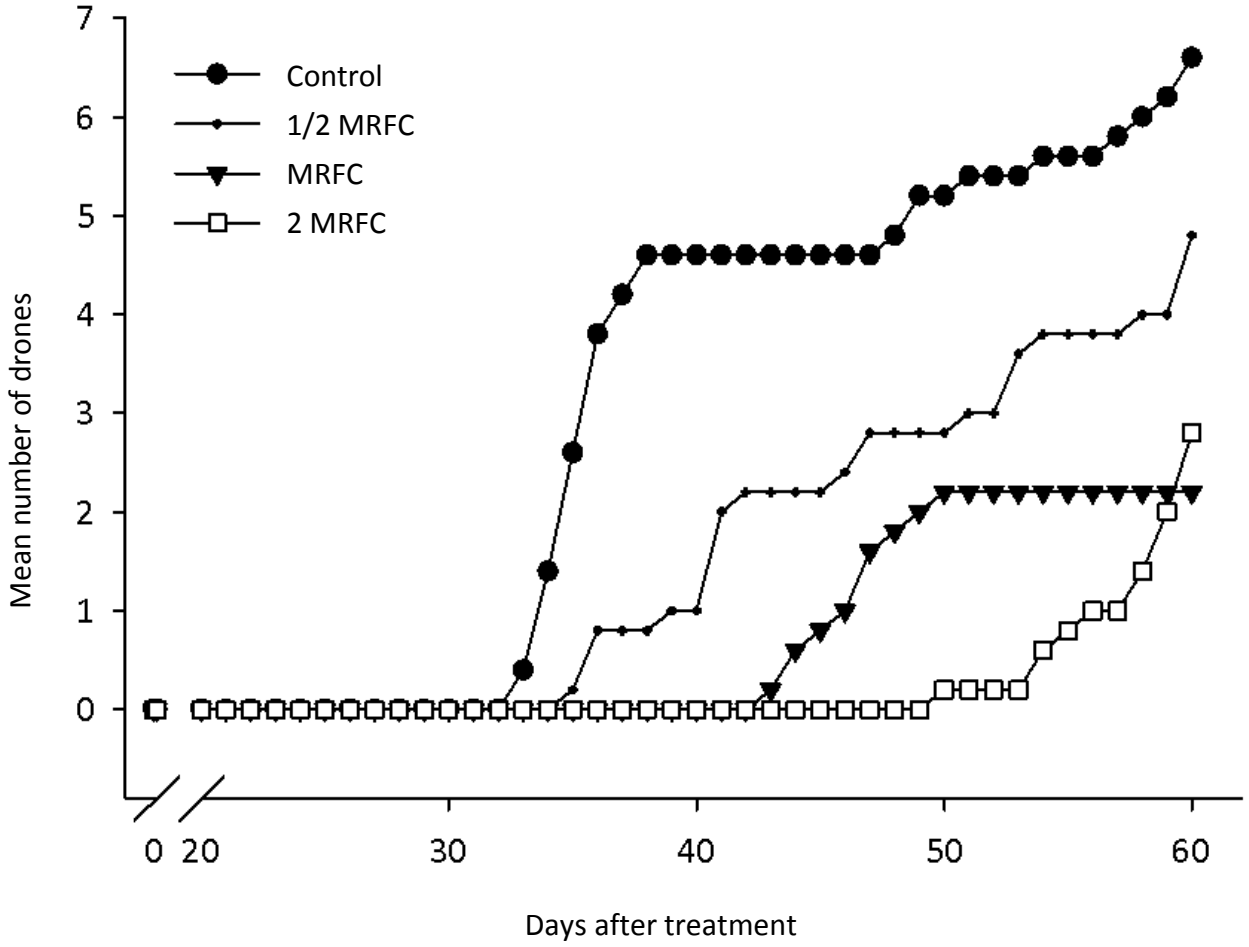

Figure 5. Drone production curves after 30 day oral exposure of *B. impatiens* workers to *B. subtilis*.

Metabolites produced by this strain in particular may have caused unexpected adverse effects in bees. It would be worthwhile examining the effects *of B. subtilis* and its metabolites (such as subtilisin, a con-specific protease) on *B. impatiens* gut flora and reproductive development (e.g. oogenesis). Toxicological assays could also be done to assess toxicity of this enzyme to the bees along with measures of titres of the protein in bees after they ingest *B. subtillis*. There are several strains of *B. subtilis* in existence, some of which occur naturally in the gut of honey bees and have no adverse effect on their host (Sabaté *et al.*, 2009).

When *B. bassiana* was administered in honey water, there was no difference in drone production due to treatment ($F = 0.82$; $df = 3, 16$; $P = 0.5014$) (Table 15). There was also no difference in the number of days to oviposition ($F = 0.62$; $df = 3, 16$; $P = 0.613$), in number of larvae ejected ($F = 1.10$; $df = 3, 16$; $P = 0.377$) nor in days to drone emergence ($F = 0.31$; $df = 3, 16$; $P = 0.819$) (Table 15) although there was a tendency for more larvae to be ejected from the control and colonies treated at 1/2 MRFC. Mommaerts *et al* (2009) did not see a significant effect on drone production when *B. terrestris* was treated orally with *B. bassiana*. The present exposure method represents worst case scenarios, where bees are coming into continuous contact with a contaminated carbohydrate source.

4.4 Conclusions:

These results show sublethal effects on *B. impatiens* of two biopesticides and a reduced risk insecticide, which have potential for use in wild blueberry pest

management programs. There was no significant mortality in any of the microcolonies for any product after worker *B. impatiens* were topically treated. *Bacillus subtilis* resulted in higher than the control rate of drone production in microcolonies where bees were treated topically with 1/2 the maximum recommended field concentration (MRFC). There were no significant reductions in drone production when *B. impatiens* was treated topically with *B. bassiana.* Spirotetramat resulted in higher drone production at 1/2 MRFC over the full MRFC but not compared with the control. There was no significantly enhanced worker mortality after oral exposure to *B. subtilis* or *B. bassiana*. Where bees were orally exposed to *B. subtilis*, drone production was significantly reduced. Oral exposure to *B. bassiana* did not result in any significant reductions in drone production. Oral exposure to spirotetramat in honey: water resulted in high mortality. The stimulation of drone production at topical exposure to treatments lower than the MRFC may be indicative of a hormetic response in the case of *B subitlis*. Oral exposure to spirotetramat in treated honey water resulted in complete mortality of treated bees compared with untreated controls. These results show that microbial based biopesticides result in complex responses that can be difficult to interpret. Exposure route and medium carrying biopesticides can play crucial roles in determining toxicity. The fact that the biopesticides are living organisms makes it difficult to assess toxicity since there are many factors affecting their growth. More work is needed to determine toxicity of biopesticides and biorational insecticides to a larger number of bee species.

CHAPTER FIVE: DISCUSSION AND CONCLUSIONS

5.1 Insecticides and Integrated Pest Management:

Insecticides continue to play a large role in the sustainable production of food. They can offer a cost-effective method of dealing with pests that exceed economic thresholds in situations where cultural and biocontrol methods may be ineffective. However, use of these products pose risks such as poisoning of fish from insecticide run-off from farms, adverse effects on applicators who work with these products, lethal or sublethal effects on non-target insects, and on rare occasions, adverse effects on consumers who consume or handle produce that may have been sprayed too close to harvest or were imported from countries with less stringent pesticide residue laws (Banken & Stark, 1998; Hamilton *et al.*, 2004; Schulz, 2004). The risk of resistance in insect pests exposed to organophosphorous, carbamate and synthetic pyrethroid insecticides is important since limited alternatives for these products exist in many agroecosystems. Governments and industry have realized many of the risks these pest control products carry, and laws concerning testing and regulation of insecticides have become more thorough and stringent. Indeed this is necessary to ensure sustainable agroecosystems and safety to consumers, producers and the environment. The government of Canada is now in the process of deregistering many organophosphorous insecticides and other older insecticides. Thus, there is a need for new "biorational" compounds that will reduce non-target impacts, reduce resistance buildup and provide greater safety to applicators and consumers. Pesticide risk to pollinators is also prevalent and can be very high depending on the system. Since pollinators are required

for about one third of produce we enjoy (Klein *et al.*, 2007), discovering new products with increased safety to pollinators is high priority.

5.2 Rationale and Summary of Current Research:

The research presented in this thesis assessed toxicity of several new bio-rational compounds that could potentially be used in the IPM of wild blueberry. Baseline toxicity of flubendiamide (Belt™ 240 SC, Bayer CropScience Canada Inc., Calgary, AB), spinetoram (Delegate™ WG, Dow AgroSciences Canada Inc., Calgary, AB), and deltamethrin (Decis 5EC®, Bayer CropScience Canada Inc., Calgary, AB) on *Itame argillacearia* Packard (blueberry spanworm) was measured as well as efficacy in controlling *I. argillacearia* populations in the field. The toxicity of biopesticide formulations of *Bacillus subtilis* Erhnberger (Cohn) (Serenade Max®, AgraQuest Inc. Davis CA) and *Beauveria bassiana* [Balsamo-Crivelli] Vuillemin (Botanigard ES®, Koppert Canada Ltd., Scarborough, ON), and the reduced risk insecticide spirotetramat (Movento®, Bayer CropScience Canada Inc., Calgary, AB) were evaluated against *Bombus impatiens* Cresson, the common eastern bumble bee.

5.2.1 Toxicity of Reduced Risk Products on *Itame argillacearia*

Although biocontrol and biotechnology control options are often desired in modern pest management, synthetic insecticides still fulfill valuable roles in the control of many pests across a wide range of agricultural systems. As resistance to conventional insecticides becomes more pronounced (Saleem *et al.*, 2008), research into more selective, environmentally friendly compounds is needed (Ishaaya, 2003). Application of

insecticides is a selection pressure and the survival and subsequent reproduction of insects that are more tolerant of products leads to evolution of resistant populations (Georghiou & Taylor, 1977). LC50 values can provide a baseline to monitor resistance development, while providing insight into the relative toxicity of different active ingredients to various populations of a pest.

There was a difference in susceptibility between the population from Nova Scotia and the one from New Brunswick in terms of susceptibility to deltamethrin but inter-population differences for spinetoram and flubendiamide were minimal. It is not unreasonable to expect that there will be differences in susceptibility between populations from different regions and this finding highlights the importance of looking at insecticide susceptibility to identify, select and implement suitable management practices. Since the susceptibility to flubendiamide and spinetoram varied minimally between populations, the potential for cross-resistance of these compounds with deltamethrin, at least in the short term, is low.

Looking at toxicity over time provided information on how long it takes the products to cause mortality at different doses. Spinetoram caused up to 100% mortality within two days at the full and 1/2 field rate. One hundred percent mortality was observed after four days of exposure for flubendiamide at the field rate but mortality did not exceed 75% for flubendiamide at 1/2 the field rate. This is consistent with what others found with these classes of compounds (Sial *et al.*, 2010; Tokumaru & Hayashida, 2010). Spinetoram is a nerve poison and thus should cause mortality more quickly than flubendiamide.

Overall, these results show that flubendiamide and spinetoram have potential in *I. argillacearia* management alongside conventional products such as deltamethrin, demonstrating excellent insecticidal activity in laboratory experiments. The LC50 values generated here will be useful in resistance monitoring of *I. argillacearia* to flubendiamide and spinetoram. Also, even though flubendiamide is slower to act, it does stop the insect from feeding, thus still providing effective crop protection.

5.2.2 Field Efficacy of Reduced Risk Products on *Itame argillacearia*

The purpose of chapter three was to assess the field efficacy of new reduced risk insecticides in controlling *I. argillacearia*. Traditionally, organophosphorous and synthetic pyrethroid insecticides were the main insecticides used for control of *I. argillacearia,* although in recent years a number of selective, safer, biorational insecticides have become available, some of which have already been registered through Canada's Minor Use Pesticide program. These include spinetoram, spinosad (Entrust®, Dow AgroSciences Inc., Calgary AB), acetamiprid (Assail®, DuPont AgroSciences Inc., Mississauga ON) and methoxyfenozide (Intrepid®, Dow AgroSciences Inc., Calgary AB) and the entomopathogenic fungus *B. bassiana* (Botanigard ES®, Koppert Canada Ltd., Scarborough, ON). Incorporation of these new products into IPM programs for *I. argillacearia* will address needs for increased environmental stewardship and can help prevent or delay the development of resistance. Selectivity of some newer products will also help in the conservation of natural enemies, pollinators and other beneficial insects of the wild blueberry system. Overall, flubendiamide, spinetoram, methoxyfenozide and acetamiprid provided control comparable to deltamethrin and

phosmet. All of the biorationals required between 4 and 10 times more product per hectare than deltamethrin but over a 100 times less product per hectare than phosmet. Population densities of *I. argillacearia* larvae were relatively low in some trials, but all products except *B. bassiana* effectively reduced the number of larvae per plot when compared with the control. Lack of efficacy of *B. bassiana* may have been due to a lack of virulence of the fungus, or strain of the fungus, towards *I. argillacearia*, suboptimal spray conditions or timing, or due to the climate of the region not being optimal for *B. bassiana* pathogenicity (Fargues *et al.*, 1997). In 2008, when pest incidence was exceptionally high, all products reduced numbers of larvae in treated plots but no difference in wild blueberry yield was seen between treated and control plots. As there are many factors that affect yield including other pest insects, diseases and weeds, this is not entirely unexpected. If more samples had been collected per plot, an effect may have been observed. Moreover, this pest can also damage blueberry plants in the sprout phase of production. Thus, if treatments are applied in the sprout year as well as the crop year, two years of feeding damage are prevented, making enhanced yield more likely than if damage is only prevented in the crop year. What growers should keep in mind is that the new biorational products do control this pest as well as conventional products but also have the added benefit of being safer to non-target beneficial insects such as bees.

5.2.3 Sublethal Effects on *Bombus impatiens*

The main objective of chapter four was to assess the sub-lethal toxicity of two biopesticides (*B. subtilis* and *B. bassiana*), and a new reduced-risk insecticide

(spirotetramat) that have potential for use in wild blueberry agroecosystems. Sublethal endpoints measured were time to oviposition, number of larvae ejected, worker survival and number of drones produced. *Bacillus subtilis* had significant effects on number of drones produced but few effects on other endpoints. However, the effects on drone production were quite variable and in some cases stimulation in drone production occurred at lower doses, particularly at the 1/2 and 1/5 maximum recommended field rate (MRFC). The stimulation was in the range of 15- 30% via topical application of *B. subtilis*. There were no significant differences of *B. bassiana* treatments and control treatments on any of the endpoints measured. Spirotetramat had no significant effect on worker mortality, number of days to oviposition and drone emergence, but had a significant treatment x time interaction effect on drone production meaning there was a difference in how fast drones were produced among treatments. There was a marginal increase of approximately 23% in number of drones produced at the 1/2 MRFC. The stimulation in number of drones produced in the *B. subtilis* and spirotetramat treatments is consistent with hormetic stimulation seen in other organisms when exposed to small amounts of a stressor that is toxic at higher doses but stimulatory at lower doses (Calabrese *et al.*, 2008; Cutler *et al.*, 2009; Guedes *et al.*, 2010). In social organisms, this phenomenon may be quite important in colony survival. This stimulation should not be interpreted as "beneficial". Further tests are warranted to examine the nature and consistency of the stimulation in insects with these agents.

Responses of *Bombus terrestris* (Linnaeus) to oral exposure of *B. subtilis* (Mommaerts *et al.*, 2009) differed from results of the current study and may be due to

species differences and subtle differences in methods. In the present study, when treated orally via treated honey water, *B. impatiens* oviposition occurred later in microcolonies treated at twice the MRFC of *B. subtilis*. So far, there have been no reports looking into how *B. subtilis* could be toxic to bees but one possibility is that by-products such as subtilin may affect the gut flora of the bees, thus impacting health and reproductive development.

It is encouraging that there was no significant difference in worker mortality between treatments except when bee microcolonies were offered spirotetramat treated honey water continuously for 30 days. In another study there was no increase in mortality when honey bee workers were offered spirotetramat-treated sugar water, but there were significant reductions in brood production (Maus, 2008) fed a spirotetramat treated carbohydrate source, and significant adverse effects on brood production were observed (Maus, 2008). This emphasizes the importance of testing products on multiple bee species since effects can be different.

In light of the declines of bee populations in recent years, the idea of testing a variety of agrochemicals on more than just one representative bee species is gaining interest, although inter-species difference in bee susceptibility to pesticides were recognized several decades ago (Johansen, 1977). The laboratory data presented here will add to the knowledge of how agrochemicals may affect bee populations.

5.3 Concluding Remarks:

The research undertaken in this thesis aimed to identify useful compounds for *I. argillacearia* management, establish baseline toxicity for two promising active ingredients with different modes of action, and to assess the toxicity of other biorational insecticides that may be used in wild blueberry pest management for *B impatiens*. In addition to showing high efficacy against *I. argillacearia,* the products tested here have potential in a variety of agricultural systems to control other lepidopteran pests. Generally, their higher specificity means fewer negative effects on beneficial non-target organisms, applicators and the environment.

Along with target specificity, managing insecticide resistance is also of prime importance in IPM. If resistance is avoided, products will retain their value in pest management and chemical inputs will be reduced overall (control failures due to resistance often result in repeated applications). Since all insecticides can potentially induce resistance, a proactive monitoring approach is important to determine baseline susceptibility to compounds. Along with continued reassessment of toxicity levels, this will permit detection of resistance early in its development.

Although there was some toxicity to pollinators with *B. bassiana*, *B. subitilis* and spirotetramat, further higher tier testing under field conditions is needed to accurately assess risks. Given the minimal effects observed in the laboratory, adverse effects on *B. impatiens* are unlikely to occur in the field. However, the toxicity of insect pathogens and agrochemicals to many non-target beneficial insects has yet to be determined. Possible hormetic responses in reproduction of bees to some of the tested microbial pesticides and reduced risk pesticides are interesting and should be investigated more

thoroughly. Stimulatory effects of pesticides are generally not considered by insect toxicologists but may have significant implications for risk assessments on beneficial insects.

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