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New options for Integrated Pest Management of *Varroa destructor* (Acari: Varroidae) in colonies of *Apis mellifera* (Hymenoptera: Apidae) under Canadian Prairie conditions

by

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Abstract

Varroa destructor Anderson & Truman 2000 (Acari: Varroidae) is an ectoparasite of *Apis mellifera* L. (Hymenoptera: Apidae) that is managed using the strategy of Integrated Pest Management to prevent *A. mellifera* colony mortality. New miticides for the Integrated Pest Management of *V. destructor* were investigated under laboratory and field conditions. The commercial miticide formulations Apollo®, Floramite®, Forbid®, and Shuttle® caused significant mortality of *V. destructor* under laboratory conditions, and are candidates for further investigation in colonies of *A. mellifera*. Field testing of miticides in colonies indicated that Apivar® and formic acid continue to provide effective *V. destructor* management, that Thymovar® use should be limited to the fall treatment window, and that alteration of the current delivery system is necessary for the new miticide HopGuard™. The results for the laboratory and field trials demonstrate the potential for new effective treatment options to supplement currently used *V. destructor* Integrated Pest Management systems.

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1. Chapter One: Literature Review

1.1. Introduction

Apis mellifera L. (Hymenoptera: Apidae), referred to as the Western honey bee (WHB), is a member of the genus *Apis* (Apidae: Hymenoptera) which comprises all species of honey bees. This genus includes the economically important *A. mellifera* and *Apis cerana* Fabr. (Asian honey bee), which are delineated from the other four extant *Apis* species by domestication and large taxonomic differences including multi-comb cavity nesting and advanced thermoregulation (Ruttner 1988). *A. mellifera* has more than 20 subspecies (Engel 1998), most of which are used in beekeeping; some subspecies are in demand as commercially superior genetic stock. *A. mellifera* is considered the leading economic honey bee species in the western hemisphere due to its ability to thrive in a spectrum of environments including harsh winters (Ruttner 1988). Pollination services provided by *A. mellifera* are vital to worldwide agro-ecosystems (Klein et al. 2007). WHBs currently experience a battery of health threats including Colony Collapse Disorder (vanEngelsdorp et al. 2009) and the ectoparasitic mite *Varroa destructor* Anderson & Trueman 2000 (Acari: Varroidae).

Varroa destructor originally parasitized only *A. cerana*, but has since transferred to *A. mellifera* colonies where it can cause damage leading to colony mortality (Rath 1999). WHBs parasitized by *V. destructor* exhibit an array of

physiological symptoms (Amdam et al. 2004). Several damaging viruses are also vectored by *V. destructor* and contribute to colony mortality (Chen and Siede 2007).

The struggle to maintain the health of WHB colonies parasitized by *V. destructor* has created the need to implement Integrated Pest Management (IPM) for *V. destructor* (Nasr and Kevan 1999, Delaplane et al. 2005). IPM for *V. destructor* includes a variety of monitoring methods, economic thresholds, and tactics to reduce *V. destructor* populations. Genetically tolerant WHBs, and miticides such as essential oils, organic acids, and synthetic miticides along with resistance management are central components of IPM for *V. destructor* (Rosenkranz et al. 2010).

1.2. Life history of the Western Honey Bee

Winston (1987) provides an extensive review of the life cycle of the WHB. Eggs hatch into larvae approximately 72 hours after being laid by the queen in a hexagonal honeycomb cell. Larvae are fed extensively by workers resulting in a large weight gain; cells are capped by workers prior to pupation. Pupae undergo a final molt before emerging from the capped cell as an adult. WHBs have a haplodiploid sex determination system wherein diploid eggs can develop into female workers or queens depending on the care they receive. Diploid eggs laid in horizontal cells and fed regular royal jelly will develop into workers. Diploid eggs fed a unique mixture of royal jelly and placed within a vertical cell will

develop into virgin queens. Haploid eggs are laid in larger cells and develop into male drones. The development time differs for the three castes: 21 days for workers, 16 days for queens, and 24 days for drones. Emerging workers are sterile females and immediately begin performing tasks within the hive; the tasks they perform will change as they age to include nest defense and foraging. Once a virgin queen emerges, she leaves the hive for mating orientation flights and subsequently mates with several drones. After mating, a queen's ovaries fully develop and egg laying is initiated. Each colony typically has only one queen which is the sole reproductive individual in the colony. Drones that have emerged will fly from the hive and seek out queens to mate with; they do not otherwise contribute to the colony.

Winston (1987) also mentions another form of reproduction that occurs in WHB colonies which is dispersal through swarming. WHB swarms contain a queen and several thousand workers; the adaptation of workers issuing with their own queen allows for swift construction of new nests and an increased probability of survival. Swarming is initiated by genetic predisposition and crowded colony conditions, which stimulate the production of additional queen cells. In preparation for a period without food stores, workers engorge themselves with honey prior to swarming. The swarm issues from the colony with the original queen once the production of additional queens is underway. The swarm moves to a suitable location and immediately begins constructing comb.

1.3. Beekeeping industry

1.3.1. Economic contribution of beekeeping

Klein et al. (2007) estimate that WHBs pollinate one-third of worldwide food crops; their contribution as managed pollinators is indispensable to world food production. It is estimated that the value of insect pollination to global agriculture is approximately $197 billion per year. In Canada the annual value of WHB contributions to pollinated crops is estimated to be $1.5 billion (Anonymous 2010). Delaplane and Mayer (2000) mention that while many pollinator-plant relationships are highly specialized*, A. mellifera* is a generalist pollinator that is effective in pollinating a wide array of crops. The WHB has become the preferred managed pollinator in the western hemisphere due to the following factors: 1) ninety-six percent of all animal-pollinated crops experience yield increases when pollinated by WHBs (Klein et al. 2007), 2) WHB colonies allocate vast resources to the collecting and storage of pollen and nectar and are able to fly up to 10 km for a rich source (Knaffl 1953), 3) the number of individuals in a WHB colony far exceeds that of other domestic pollinators (Delaplane and Mayer 2000), and 4) the development of movable hives as well as intensive colony management facilitate large scale movement of colonies. Continued maintenance of healthy colonies is important as increased demand for pollination and concurrent decline of natural pollinators is likely to put additional pressure on managed WHB colonies to overcome the pollination deficit (Aizen and Harder 2009).

In Canada, WHB colonies are rented to pollinate fruit trees, berries, melons and squashes, canola, legumes, and forage crops (Anonymous 2010). Within Alberta, WHB colonies are frequently employed to pollinate pedigreed hybrid seed canola, which is the main source of seed for the hybrid canola cultivars grown for oil production across the Canadian prairies (Canola Council of Canada 2012).

Although the chief contribution of *A. mellifera* to agriculture is undeniably pollination services, WHBs also produce many highly valued products. In 2011 Canada produced approximately 35.4 million kilograms of honey valued at approximately $151 million (Statistics Canada 2012). WHBs also produce beeswax, used in candles and cosmetic merchandise. Various other hive products are also sold as natural health products such as propolis: a substance bees collect from tree resins with uses in traditional medicine; pollen: a protein source frequently used as a dietary supplement; and royal jelly: the substance fed to larval queens.

1.3.2. Health threats to the Western Honey Bee

WHBs are foundational to worldwide agro-ecosystems. Therefore, threats to WHB health jeopardize the stability of the world's food supply. Serious health threats to *A. mellifera* include bacteria, microsporidians, viruses, colony disorders, and parasitic mites; these threats have contributed to the loss of most wild colonies of WHBs (Kraus and Page 1995). Intensive management is required

to maintain healthy WHB colonies undamaged by an array of health threats.

WHB larvae are vulnerable and susceptible to American foulbrood (AFB), a bacterial infection caused by *Paenibacillus larvae* (Shimanuki et al. 1992). Spores are ingested by young larvae, germinate in the midgut, and eventually protrude through the lining of the gut, producing a septic condition which causes the larvae to die. Left untreated, AFB will cause colony mortality. Spores of *P. larvae* can persist indefinitely in beekeeping equipment and cause reinfection. AFB is visually diagnosable by trained beekeepers and treatments for it include the incineration or radiation of all infected hive equipment, or the treatment of the colony with a registered antiobiotic. In Canada, AFB is treated with oxytetracycline. In some cases tylosin may be used when oxytetracycline resistance has been shown (Thompson et al. 2007).

Nosemosis is an intestinal disease caused by the microsporidia *Nosema apis* and *Nosema ceranae* and is reviewed by Fries (1997). Adult WHBs ingest the spores and the microsporidia multiply in the epithelial cells of the midgut. Spores are voided with feces and will infect house cleaning WHBs. Individuals infected with *Nosema* spp. have shorter life spans, impaired ability to act as nurse bees, and generally experience behavior changes. Nosemosis is particularly damaging in northern climates because the long cold winters confine WHBs to the hive which increases contact between individuals. Monitoring for Nosemosis is difficult as evidence for infection can only be diagnosed through microscopic examination, a situation that few beekeepers are equipped to do. Recent

evidence suggests that *N. ceranae* (which transitioned to *A. mellifera* from its original host *A. cerana)* is more prevalent than *N. apis* among North American WHB populations (Chen et al. 2008). Fumagillin-B is used to treat Nosemosis in Canada and has remained effective since its registration (Williams et al. 2008).

At least 18 separate viruses are found to infect WHBs (Chen and Siede 2007). While some of these viruses manifest in obvious symptoms such as Deformed wing virus (DWV), Black queen cell virus (BQCV), or Sacbrood virus (SBV); other viruses such as Kashmir bee virus (KBV), Israeli acute paralysis virus (IAPV), Acute bee paralysis virus (ABPV), and Chronic bee paralysis virus (CBPV) are not visually apparent (Chen and Siede 2007).

Perhaps the most widely known threat to WHBs is Colony Collapse Disorder (CCD). The symptoms of CCD are described by vanEngelsdorp et al. (2009) as follows: large patches of brood with a queen present, insufficient workers remaining to maintain the amount of brood, and no dead WHBs in the hives or apiary. Substantial colony losses experienced in the USA in the winters of 2006-2008 were attributed to CCD (vanEngelsdorp et al. 2009). Research initiatives have not been able to identify the causative agent of CCD, but an array of contributing causes has been suggested including stress from long distance colony movement for pollination, *N*. *ceranae* infections, Israeli Acute Paralysis Virus (vanEngelsdorp et al. 2009), and chronic effects of pesticide residues in WHB colonies (Mullin et al. 2010). At present there is no evidence for the occurrence of CCD in Canada (Kevan et al. 2007; Guzman-Novoa et al. 2010).

Varroa destructor is an ectoparasitic mite of *A. mellifera* and has become a major threat to the health of worldwide managed WHBs (Rosenkranz et al. 2010). *Varroa destructor* will subsequently be discussed in greater detail.

1.4. Biology of *Varroa destructor*

Like many parasites, *V. destructor* has a balanced relationship with its original host, *A. cerana,* which does not normally lead to colony death (Rath 1999). Unlike *A. mellifera, A. cerana* colonies have adapted to co-exist with *V. destructor* without colony mortality. *Varroa* spp. on *A. mellifera* were originally thought to be *Varroa jacobsoni* Oudmans until Anderson and Trueman (2000) determined that it was a separate species which they named *Varroa destructor. V. destructor* is thought to have shifted from *A. cerana* to *A. mellifera* during the 1950s; it is suggested that this host shift occurred in both Japan and Russia (Oldroyd 1999). Subsequently, *V. destructor* quickly spread throughout the world's populations of *A. mellifera*, reaching South America in 1971 (Oldroyd 1999), and North America in 1987 (De Guzman and Rinderer 1999). Currently, *V. destructor* is ubiquitous to most countries with WHBs, with the notable exception of Australia (Rosenkranz et al. 2010).

1.4.1. Life history of Varroa destructor

Martin (2001) separates *V. destructor* life history into the phoretic phase during which *V. destructor* are attached to and feeding on adult WHBs and the reproductive phase during which *V. destructor* are within a capped cell and are

reproducing and feeding on the developing pupae. It should be noted that the term “phoretic” is consistently but incorrectly applied to *V. destructor.* Phoresy implies a non-parasitic relationship for the purpose of transportation; the relationship between WHBs and *V. destructor* is undoubtedly parasitic. Nevertheless, the term has persisted and is standard terminology within WHB literature.

The *V. destructor* life cycle is summarized in Figure 1.1. During the phoretic phase, *V. destructor* are feeding on adult WHBs, quite often between the abdominal plates where they are protected and not easily dislodged (Martin 2001). When *V. destructor* on WHBs pass by a suitable brood cell that is soon to be capped, they move off the WHB, enter the cell, and are submerged in the brood food where they respire through erect snorkel-like peretrimes. After the cell is capped and the larva has consumed all the brood food, the *V. destructor* begins feeding on the developing WHB pupa. Approximately 70 hours after capping, the *V. destructor* lays its first egg unfertilized which will hatch as a haploid male, and then all subsequent eggs laid (in 20 hour intervals) are diploid females. After the nymphs have matured into adults, the offspring in the cell mate (sibling mating). Once the WHB is mature, it emerges from its cell and all mature female *V. destructor* leave the cell as well. Immature females and males of *V. destructor* die in the cells. Newly emerged mature female *V. destructor* spend time in the phoretic phase before entering a cell to reproduce. Time spent in the phoretic phase varies greatly. At the peak of summer it may be very short

or non-existent whereas in winter it might last several months because of the broodless period. The timing and duration of the phoretic phase is important because it is the target of most available *V. destructor* management methods. *V. destructor* shows a marked preference (8-10 times) for drone brood over worker brood as drone pupae are larger, and development time is longer, thus allowing for more *V. destructor* progeny in each cycle (Martin 2001).

As *V. destructor* are unable to live on hosts other than WHBs, the introduction of *V. destructor* to an area or colony can only be facilitated by the movement of WHBs. Colonies are infested by phoretic *V. destructor* on workers or drones of WHBs drifting from colony to colony. *V. destructor* also spreads by way of WHB swarms from infested colonies, as well as the artificial transfer of combs between colonies by beekeepers (Winston 1987).

1.4.2. Effects of Varroa destructor parasitism

WHBs parasitized by *V. destructor* exhibit a variety of physiological symptoms. WHBs parasitized as pupae may have lower emerging weights (De Jong et al. 1982a), impaired organ development (Schneider and Drescher 1987) and suppressed immune systems (Yang and Cox-Foster 2007). Parasitized adult WHBs are more likely to become disoriented during flight and not return to the hive (Kralj and Fuchs 2006) and are less likely to survive the winter (Amdam et al. 2004). In heavily infested colonies, brood irregularities known as parasitic mite syndrome (PMS) might also be evident (Shimanuki et al. 1994).

V. destructor is a highly efficient vector of viruses that are harmful to WHBs. Almost all WHB viruses are vectored by *V. destructor*, with the likely exception of CBPV (Chen and Siede 2007). Many viral infections go unnoticed in WHBs; the most obvious is DWV, which is frequently found in colonies with high *V. destructor* infestations and manifests itself in shriveled or bent wings on WHBs (de Miranda and Genersch 2010). Remarkably, DWV is capable of replicating in *V. destructor* tissues, thus increasing the quantity of DWV that *V. destructor* can vector and spread in WHB colonies (de Miranda and Genersch 2010). Research has shown that *V. destructor* infestations in conjunction with DWV loads reduce the lifespan of WHBs (Dainat et al. 2012) and infection with DWV is associated with colony mortality (Highfield et al. 2009).

The relationship between *A. mellifera* and *V. destructor* has substantially different implications in temperate regions than tropical ones. This is largely due to a broodless period that exists during the winter in temperate climes (Winston 1987). This period confers both an advantage and a disadvantage in the control of *V. destructor*. It is advantageous because *V. destructor* is confined to the phoretic phase, hence limiting reproduction and facilitating control measures directed at the phoretic phase (Rosenkranz et al. 2010). However, the WHB colony population also declines rapidly before and during the winter (Winston 1987), which results in more *V. destructor* in the colony per WHB. Additionally, colonies overwintering successfully require a good generation of long lived "winter bees" which may survive 6 months or more (Amdam et al. 2004). Winter

bees produced in a colony heavily infested with *V. destructor* are less likely to survive for this amount of time (Amdam et al. 2004; Dainat et al. 2012). These considerations likely result in *V. destructor* causing more colony mortality in temperate regions rather than tropical ones (Rosenkranz et al. 2010).

1.5. Integrated Pest Management of *Varroa destructor*

The struggle to maintain WHB health in the wake of *V. destructor* has created the need to educate beekeepers about the benefits of using Integrated Pest Management (IPM) to manage *V. destructor* (Nasr and Kevan 1999). Luckmann and Metcalf (1982) show that generations of preventative applications of pesticides have resulted in the need for IPM in agricultural systems. They outline six steps of IPM which can be summarized as (1) pest identification, (2) knowledge of biology, (3) monitoring of population levels, (4) determination of treatment thresholds, (5) tactic selection, and (6) evaluation of results. IPM theory can be applied to the practical management of *V. destructor* by beekeepers; typically the areas of monitoring, thresholds, and tactic selection are emphasized. Tactics include the use of genetically tolerant WHBs, and control methods such as essential oils, organic acids, and synthetic miticides.

1.5.1. Timing of Integrated Pest Management for Varroa destructor

Before implementing IPM, it is important to consider seasonal timing and the state of the colony (Delaplane 1998). Choices made regarding monitoring methods, economic thresholds, and tactic selection may vary depending on the

season. Winter inaccessibility to hives, honey production, and pollination demands often create narrow windows during which beekeepers are able to monitor and treat their colonies. Additionally, control tactics are more effective when colonies are broodless, which may also be a consideration when managing *V. destructor* (Ellis et al. 2009). Therefore, monitoring methods and economic thresholds for treatment are more effective when seasonally and regionally specific (Strange and Sheppard 2001). In Alberta, there are two narrow treatment windows during which beekeepers are able to access and apply treatments to their colonies. These windows are in late spring (April-June) after the colonies emerge from winter and prior to honey production, and in early fall (Late August - November) after the honey flow and before the onset of winter (Figure 1.2).

1.5.2. Monitoring methods for Varroa destructor

Effective IPM for *V. destructor* in WHB colonies must involve monitoring of the *V. destructor* population levels. Lack of proper monitoring often leads to prophylactic use of miticides by beekeepers (Hood and Delaplane 2001). Additionally, field-based monitoring methods are necessary to encourage beekeepers to monitor and to facilitate prompt management decisions. Current monitoring practices involve assessment of the number of phoretic *V. destructor* on a sample of WHBs from the colony or monitoring natural *V. destructor* mortality within a colony.

There are several monitoring methods that estimate the number of *V. destructor* on a subsample of the adult WHB population (usually 300 WHBs). These methods include the sugar roll, the ether roll, the alcohol wash, and the alcohol wash using the *Varroa* Hand Shaker. For each of these methods, the number of *V. destructor* counted on the WHBs is divided by the total number of WHBs in the sample and expressed as *V. destructor* infestation. The methods vary simply in the manner in which the *V. destructor* are separated from the WHBs and counted.

The sugar roll is performed by collecting approximately 300 WHBs in a jar covered by a screen, and then coating the WHBs with icing sugar. The icing sugar stimulates WHBs to mechanically groom themselves and dislodged phoretic *V. destructor* fall through the screen when the jar is inverted and shaken (Macedo et al. 2002).

Approximately 300 WHBs are collected in a sealed jar for the ether roll method. The WHBs are sprayed with diethyl ether, and then the jar is shaken. Dislodged *V. destructor* stick to the sides of the jar and are counted (Shimanuki and Knox 1987).

The alcohol wash method involves preserving a sample of 300 WHBs in alcohol so that *V. destructor* are killed. The sample is shaken and then rinsed repeatedly through a size-specific strainer so that *V. destructor* fall through and are counted (De Jong et al. 1982b). A quicker version of the alcohol wash can be performed with the *Varroa* Hand Shaker (Nasr and Williamson 2010).

Another method of monitoring *V. destructor* is to assess the natural mortality of *V. destructor* within colonies. Colonies with larger *V. destructor* populations generally exhibit increased natural mortality of *V. destructor.* A screened bottom board is placed beneath the hive that allows dead *V. destructor* to fall through but not WHBs. A sticky trap is inserted below the screen and the number of *V. destructor* counted at regular intervals and expressed as *V. destructor* mortality per day (Shimanuki and Knox 1987).

1.5.3. Economic thresholds for Varroa destructor

The use of economic thresholds to manage of *V. destructor* in WHB colonies discourages prophylactic use of miticides and enables judicious decision making by beekeepers (Strange and Sheppard 2001). Treating only when warranted is cost-effective and serves to reduce the selective pressure of miticides (Delaplane et al. 2005). Considering the narrow range of effective miticides available, it is necessary to ensure that each available miticide is effective for as long as possible. As geographic differences in *A. mellifera* seasonal activities and brood rearing periods are likely to have corresponding effects on *V. destructor* population, the development of regionally specific thresholds is necessary (Delaplane 1998). When miticides are used in accordance with treatment thresholds, the time between chemical treatments can be delayed, and the risk of resistance development is lowered (Strange and Sheppard 2001).

Economic thresholds for *V. destructor* treatment have been put forth for the southeastern United States (Delaplane and Hood 1999), Washington State (Strange and Sheppard 2001) and the Canadian Prairies (Currie and Gatien 2006; Nasr et al. 2008). Published thresholds (Table 1.1) vary widely according to region, with higher thresholds observed in southern climates, and more conservative thresholds established for northern climates. A longer active season and greater number of brood cycles necessitates two yearly miticide treatments in southern climates (Delaplane and Hood 1999) which results in higher thresholds for treatment. In more northerly climates, one yearly miticide treatment is typically sufficient, thus lower thresholds for treatment are observed (Strange and Sheppard 2001; Currie and Gatien 2006; Nasr et al. 2008). Furthermore, the risk associated with long, cold winters that cause lengthy lulls in the WHB brood cycle, WHB population dwindling, as well as narrow treatment windows also contribute to the more conservative thresholds suggested by Currie and Gatien (2006) and Nasr et al. (2008).

1.6. Tactic selection for Integrated Pest Management of *Varroa destructor*

Once it is the appropriate time to treat, colonies have been monitored, and the *V. destructor* population is above the economic threshold, a tactic to reduce the *V. destructor* population below the threshold is necessary. Several tactics are available; they include using genetically tolerant WHBs, and employing treatments such as essential oils, organic acids, and synthetic

miticides (Rosenkranz et al. 2010). It is important to consider which sub-population of *V. destructor* is targeted by a *V. destructor* management tactic (Meikle et al. 2012). As *V. destructor* in the reproductive phase within capped cells are protected from most treatments, treatments targeting phoretic *V. destructor* only may be insufficient to reduce the population below the economic threshold (Calderone 2010). For instance, Ellis et al. (2009) found that approximately 60% of the *V. destructor* in a colony were in the reproductive phase; therefore short term treatments capable of causing >90% mortality of phoretic *V. destructor* at the time of treatment only caused mortality of 36% of the total *V. destructor* within a colony. Therefore, effective treatments need to have residual activity to cause mortality of *V. destructor* as they emerge from the reproductive phase, or alternatively need to be applied several times (Giovenazzo and Dubreuil 2011). However, it should be noted that short term treatments can be effective during broodless periods when all the *V. destructor* are in the phoretic phase, and therefore exposed to the treatment.

1.6.1. Genetically tolerant strains of Apis mellifera

Rothenbuhler (1964) published the first finding of a direct genetic basis for behavior, while studying the mechanisms for AFB resistance in WHB colonies. Rothenbuhler coined the term “hygienic behavior” which he found was determined by two recessive genes governed by Mendelian inheritance. One locus was associated with workers uncapping the infected cell, and the second

locus was associated with removal of the diseased larva. Rothenbuhler's work was foundational for the field of behavioral genetics and for *A. mellifera* genetics in particular.

What has arisen from Rothenbuhler's (1964) work is the idea of breeding strains of WHBs that are tolerant to diseases or pests. Spivak and Boecking (2001) outline four inherent difficulties that arise when breeding resistance into WHBs. Firstly, it must be ascertained what the particular mechanisms are that make a colony more or less resistant to *V. destructor* infestations. Secondly, the heritability of these mechanisms needs to be established. Thirdly, once a suitable line has been found, it must be propagated, maintained, and distributed commercially. Finally, there is frequent disparity between the definition of resistance and the goals for breeding resistance in *A. mellifera.* Le Conte et al. (2007) also show that WHB strains that display tolerance to *V. destructor* may lose valuable economic traits such as honey production.

Selection for *V. destructor* tolerance by *A. mellifera* is expedited by an available model of tolerance in *A. cerana.* In contrast to *A. mellifera, V. destructor* reproduction rarely occurs within *A. cerana* worker cells, with the bulk of the reproduction occurring within drone brood (Rath 1999). Furthermore, drone brood has a thicker capping that a drone weakened by several *V. destructor* cannot penetrate, thereby creating a trap during high infestations (Rath 1999). Additionally, *A. cerana* exhibits grooming and removal behaviors, both of which result in reduced *V. destructor* populations (Peng et al. 1987). Of

these adaptations, two are behavioral: grooming behavior and removal behavior. Grooming behavior includes auto-grooming where WHBs remove *V. destructor* from themselves, and allo-grooming where *V. destructor* are removed by nest mates (Peng et al. 1987). Removal behavior by *A. cerana* results in workers removing pupae infested with *V. destructor* from their cells (Rath and Drescher 1990). There are indications that grooming and removal behaviors do exist in *A. mellifera,* but in a diminished capacity compared to *A. cerana* (Spivak and Boecking 2001).

Removal of pupae infested with *V. destructor* by *A. mellifera* is likely similar to other forms of hygienic behavior in WHBs involving the removal of diseased larvae (Boecking and Spivak 1999). A different form of hygienic behavior has been described as *Varroa* Sensitive Hygiene by Harris (2007). Harris (2008) later found an additional component of *Varroa* Sensitive Hygiene whereby *V. destructor* are removed from a cell but the pupae remain and continue developing. Commercial stocks of *A. mellifera* expressing *Varroa* Sensitive Hygiene are available and have generally displayed decreased *V. destructor* population growth in comparison to controls while retaining economic traits (Reviewed by Rinderer et al. 2010). Delaplane et al. (2005) show that current IPM practices such as the use of hygienic WHB stocks are not sufficient to eliminate miticide use, but can be used to delay time between miticide treatments, thus reducing chemical exposure in the hive, and lengthening the time before resistance development to applied miticides.

1.6.2. Essential oils

More than forty-two essential oils from plant extracts have been screened for miticidal activity against *V. destructor* (Reviewed by Umpierrez et al. 2011). Essential oils with ability to serve as *V. destructor* control agents include Chamomile oil, clove oil (Umpierrez et al. 2011), menthol, camphor, and thymol (Imdorf et al. 1999). Thymol has been adopted widely as a *V. destructor* treatment, and may be used on its own, or in blends with other essential oils (Imdorf et al. 1999). Various homemade formulations incorporating thymol have been used (Imdorf et al. 1999), and commercial formulations such as Api Life VAR®, Thymovar® and Apiguard® are available (Rosenkranz et al. 2010). Currently, thymol and the commercial miticide Thymovar® are registered for use in Canada (PMRA 2010b).

An important consideration when applying thymol-based products is the ambient temperature; most thymol products require an ambient temperature range of 15-20°C to be effective (Imdorf et al. 1995). Calderone (1999) found that the evaporation of a thymol-blend was positively correlated with temperature. This finding was further confirmed by Emsen et al. (2007) who showed that *V. destructor* mortality was correlated with temperature when using thymol products. Rosenkranz et al. (2010) suggest that the relationship between temperature and evaporation of essential oils within the colony leads to the variability observed in *V. destructor* mortality when essential oils are used. Thymol-based products have been associated with side effects within WHB

colonies (Floris et al. 2004). Ensuring adequate evaporation of thymol in the hive without causing WHB mortality has proven to be an obstacle to thymol-based *V. destructor* management (Imdorf et al. 1999).

A wide range of efficacies have been reported for thymol products. They range from 97% for Thymovar® (Baggio et al. 2004), to 83% for thymol dusts and 76% for thymol in vermiculite blocks (Emsen et al. 2007). Calderone (1999) reported 70% for a blend similar to Api Life VAR®. Reported efficacies for the gel-based Apiguard® include 76% (Matilla and Otis 2000) and 46% (Gregorc and Planinc 2005).

1.6.3. Organic acids

A variety of organic acids have been successfully used to manage *V. destructor.* The most prevalent are formic acid and oxalic acid. Additionally, organic acids extracted from hop plants have recently been investigated for *V. destructor* activity (DeGrandi-Hoffman et al. 2012).

65% Formic acid and commercial formulations are registered in Canada (PMRA 2005). Formic acid can be applied directly to the bottom board of the colony (Giovenazzo and Dubreuil 2011), or incorporated within an absorbent pad (Nasr et al. 2008) or gel matrix (Kochansky and Shimanuki 1999). Formic acid is the only known *V. destructor* treatment that is capable of killing *V. destructor* within capped cells (Fries 1991; vanEngelsdorp et al. 2008). Due to evaporation, formic acid requires a range of ambient temperatures of 12-25°C to achieve

optimum *V. destructor* mortality (Wallner and Fries 2003).

Studies report efficacy ranges between 50-80% for single formic acid treatments; efficacy can be increased by several treatments over a few weeks or continuous delivery systems (Calderone 2000). Formic acid fumes can kill developing brood and WHBs (Elzen et al. 2004; Giovenazzo and Dubreuil 2011) or cause queen mortality (Giovenazzo and Dubreuil 2011; Underwood and Currie 2007) when applied to colonies so careful use is important. Due to inconsistent efficacy of treatments, and difficulties with treatment timing, formic acid is unreliable as the sole *V. destructor* treatment (vanEngelsdorp et al. 2008). However, formic acid is ideal as part of an IPM program as it can supplement mortality caused by a synthetic miticide, and manage resistance to currently used synthetic miticides.

Oxalic acid was approved by PMRA in Canada in 2005 for management of *V. destructor* (PMRA 2010a). Rademacher and Harz (2006) extensively reviewed the three methods of applying oxalic acid: it can be sprayed, trickled, or sublimated within the colony. As oxalic acid only causes mortality of phoretic *V. destructor* at the time of application, they recommend it is best applied during broodless periods. They found that it tends to be well tolerated by WHBs, and often results in over 90% *V. destructor* mortality when used during broodless periods. The efficacy of oxalic acid is greatly decreased when brood is present in colonies. Oxalic acid can be used as the sole *V. destructor* management product, or in conjunction with a synthetic miticide to manage resistance.

1.6.4. Synthetic miticides

In Canada three synthetic miticides are currently registered for *V. destructor* control. Apistan® contains the active ingredient fluvalinate which is a pyrethroid; it was registered in 1994 (PMRA 1994) and was widely used until *V. destructor* developed resistance to it in 2001 (Currie et al. 2010). Checkmite+™ contains the organophosphate coumaphos, was approved for use in 2003 (PMRA 2008) and provided good control until *V. destructor* became resistant (Currie et al. 2010). Apistan® and Checkmite+™ were both controversial miticides as they were associated with lower quality queens (Haarmann et al. 2002) sterile drones (Rinderer et al. 1999; Burley et al. 2008) and high wax residues (Martel et al. 2007). Apivar® contains the active ingredient amitraz, which belongs to the novel class of formamidines. Apivar® was first registered for emergency use in 2008 (PMRA 2009) and is currently very effective in managing *V. destructor* infestations (Nasr et al. 2010). Additionally, Apivar® has been associated with much lower residues in wax than its predecessors (Martel et al. 2007). Apistan®, Checkmite+™, and Apivar® have similar application methods; these three miticides are applied to colonies in plastic strips impregnated with the miticide. Phoretic *V. destructor* are killed through contact to the miticide strips; the strips are left in for six weeks to kill *V. destructor* emerging from successive brood cycles (Ellis 2001).

Despite the risks associated with using synthetic miticides, they remain the only consistent method of managing *V. destructor* populations to below the

economic threshold (Delaplane et al. 2005). Other control tactics such as genetically tolerant WHBs, essential oils, and organic acids are able to delay time between miticide treatments, but are not sufficient to maintain colonies free from *V. destructor* damage (Delaplane et al. 2005).

1.7. *Varroa destructor* resistance to synthetic miticides

Managing resistance to synthetic miticides is a fundamental component of IPM systems for *V. destructor*. A side effect of using synthetic miticides is that they have the potential to quickly become useless as the population of *V. destructor* becomes resistant (Milani 2001). Miticides create a bottleneck wherein only *V. destructor* that possess a mutation allowing them to survive and increase their fitness. Considering the reproductive ability of *V. destructor* (Martin 1998), this bottleneck can quickly recover to a resistant population at the level it was prior to miticide application.

Resistance development in *V. destructor* is more likely to occur than in other arthropods due to several reasons: 1) Sammataro et al. (2005) suggest that inbred sibling matings of *V. destructor* in conjunction with haplodiploidy likely expedites resistance development; 2) although resistance development is normally expected to come at a cost to fitness, studies have shown that *V. destructor* fitness does not decrease when resistance to a miticide (Apistan®) has developed (Martin et al. 2002); 3) synthetic miticides are typically left in the colony for six weeks, which provides an extended period of selection pressure; 4)

synthetic miticides tend to leave residues in wax which accumulate with each treatment, thereby ensuring that the *V. destructor* are continually exposed to the miticide (Milani 2001); 5) the same miticide is generally used for several years in a row without alternation, and sometimes without other IPM methods; and 6) modern pollination regimes requiring the movement of WHB colonies throughout countries also increases the likelihood of spreading existing resistance to previously unaffected areas.

Table 1.2 summarizes the spread of *V. destructor* resistance development to commonly used miticides throughout the world. While resistance to coumaphos and amitraz also exists, only the mechanisms for resistance to fluvalinate are well established (Van Leeuwen et al. 2010). Resistance to fluvalinate can be conferred through metabolic changes (Hillesheim et al. 1996), but also can be mediated through target-site mutations (Wang et al. 2003).

An integral part of IPM is managing resistance development. Resistance management for *V. destructor* involves monitoring and economic thresholds so that miticides are only used when necessary rather than prophylactically (Strange and Sheppard 2001). Additionally miticides need to be used according to label recommendations; varying the miticide concentration, duration of application, or timing of application can lead to premature resistance development. Finally, the use of IPM tactics such as genetically tolerant WHBs, essential oils, and organic acids in conjunction with synthetic miticides can delay resistance development (Milani 2001).

1.8. Research objectives:

Considering the resistance issues with all currently approved miticides, and the inability of non-chemical methods to reliably manage *V. destructor* infestations, new management tactics need to be developed and assessed for treatment of *V. destructor* on WHBs. The development of new tactics is crucial to maintaining healthy WHB colonies until sustainable permanent solutions for *V. destructor* management can be found and implemented (Dietemann et al. 2012).

Developing new effective control tactics for *V. destructor* will require extensive laboratory and field screening. Chapter two outlines the laboratory evaluation of new synthetic miticides unrelated to those currently used against *V. destructor*. Miticides were screened in the laboratory using a glass vial bioassay to determine the LC_{50} for *V. destructor.*

Chapter three describes the field application of non-synthetic miticides not previously tested in Alberta: the essential oil Thymovar®, and the organic acid HopGuard™. Field methods were rigorous to establish any side-effects from the applied miticide. Efficacy on *V. destructor* was determined in comparison to currently used industry standards and the miticides were evaluated within the conditions and treatment windows of Alberta.

Chapter four provides a synthesis of this research and presents recommendations on how it can be incorporated into existing IPM systems. It also contains suggestions for the improvement of currently used IPM systems.

Tables

Table 1.1. Location, time, and method-specific economic thresholds for *V. destructor* management from recent publications. The treatment thresholds are summarized from original publication to *V. destructor* infestation (calculated with the ether roll or the alcohol wash methods) or natural *V. destructor* mortality per day using sticky traps.

Reference	Location, Time	Infestation (ether roll method)	Infestation (alcohol wash method)	Natural mortality (sticky trap)
Delaplane and Hood 1999	South-eastern United States, August	5-13%		59-187 per day
Strange and Sheppard 2001	Washington, April	1%		12 per day
	Washington, August	4.7%		23 per day
	Washington, October	1%		
Currie and Gatien 2006	Manitoba, April		2%	
	Manitoba, August		4%	
	Manitoba, late fall		12%	
Nasr et al. 2008	Alberta, April		3%	>20 per day
	Alberta, Fall		1%	10-20 per day

Table 1.2. Timeline of years when resistance was reported throughout the world to fluvalinate, coumpahos, and amitraz.

	Fluvalinate (Apistan®)		Coumaphos (Checkmite+™)		Amitraz (Apivar®)	
Location	Year Reported	Reference	Year Reported	Reference	Year Reported	Reference
Alberta	2001	(Currie et al. 2010)	2006	(Currie et al. 2010)	Not yet reported	
Canada	2001	(Currie et al. 2010)	2002	(Currie et al. 2010)	Not yet reported	
USA	1998	(Elzen et al. 1998)	2002	(Elzen and Westervelt 2002)	2000	(Elzen et al. 2000)
UK	Not yet reported		2002	(Thompson et al. 2002)	Not yet reported	
Italy	1995	(Lodesani et al. 1995)	Not yet reported		Not yet reported	
Argentina	1997	(Fernandez and Garcia 1997)	2008	(Maggi et al. 2009)	2010	(Maggi et al. 2010)
Mexico	Not yet reported		Not yet reported		2005	(Roríguez-Dehaibes et al. 2005)

Figures

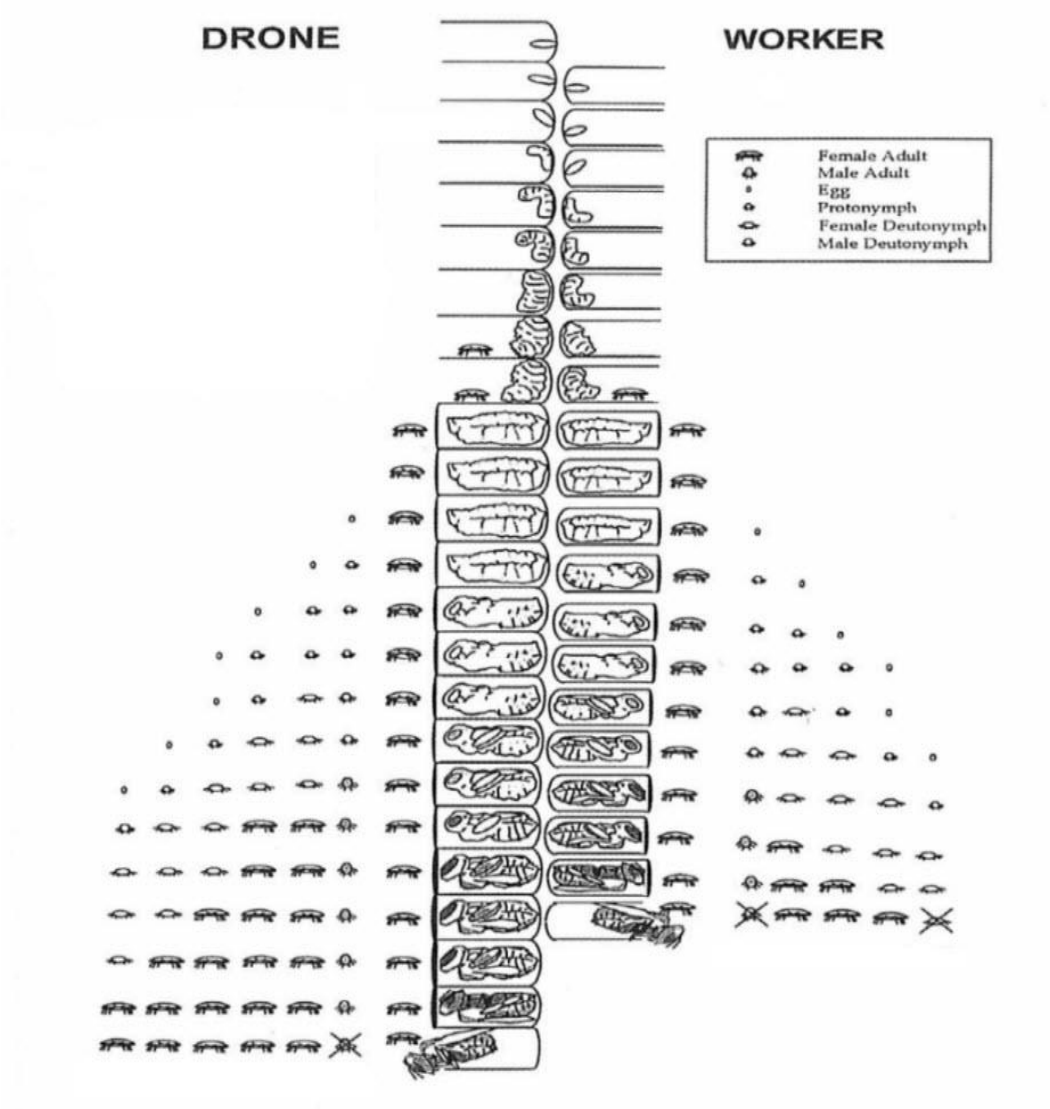

Figure 1.1. The development of *Varroa destructor* within drone brood and worker brood of *Apis mellifera.* From Martin (2001). Used with permission.

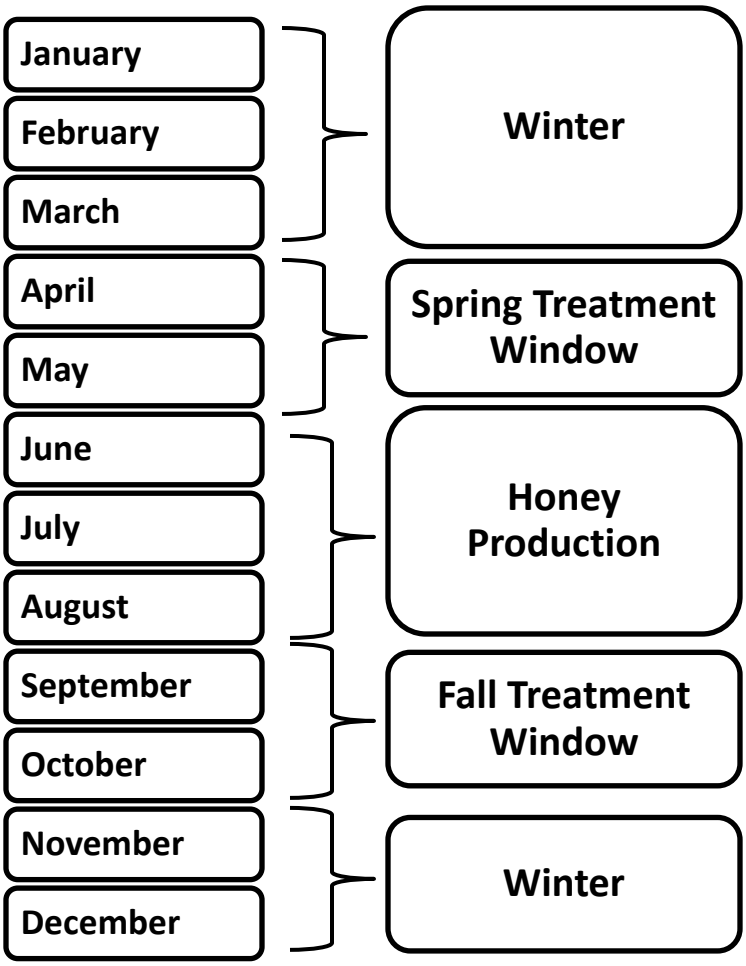

Figure 1.2. Calendar of conditions for managment and treatment of WHB colonies for *V. destructor* within Alberta. "Winter" indicates the months during which WHB colonies are inaccessible. "Spring Treatment Window" refers to the time in spring when colonies are accessible and can be treated for *V. destructor. "*Honey Production" indicates the months during summer when colonies cannot be treated because honey is being collected. "Fall Treatment window" refers to the time in fall when colonies can be treated for *V. destructor* prior to the onset of winter.

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2. Chapter Two: Comparative susceptibility of *Varroa destructor* (Acari: Varroidae) to five miticides under laboratory conditions

2.1 Introduction

The Western Honey Bee (WHB), *Apis mellifera* L. (Hymenoptera: Apidae), plays a role of fundamental importance to the agricultural economy of most countries. While the hive products garnered by beekeeping are valuable, the contribution of WHB to pollination of agricultural crops far exceeds the value of hive products (Aizen and Harder 2009). As many crops are reliant upon WHBs for yields, or maximize yields following cross pollination, the successful management of healthy WHBs is indispensable to global food production (Gallai et al. 2009).

Varroa destructor Anderson and Trueman 2000 (Acari: Varroidae) parasitizes pupal and adult stages of WHBs and is an effective vector of several harmful viruses (Chen and Siede 2007). Without adequate management, *V. destructor* can cause irreparable harm to healthy WHB colonies often resulting in colony mortality. Integrated Pest Management (IPM) including judicious application of efficacious synthetic miticides is necessary for maintaining healthy colonies undamaged by *V. destructor* (Delaplane et al. 2005). Synthetic miticides for *V. destructor* have included organophosphates, pyrethroids, and formamidines, but resistance to these groups has occurred (Pettis 2004, Elzen et al. 2000). Several factors promote early acquisition of *V. destructor* resistance to synthetic miticides including (1) *V. destructor* life history, specifically inbred

haplo-diploid mating; (2) long miticide application periods along with miticide residues in wax; and (3) the use of the same miticide in successive years without rotation or other IPM tactics. Despite IPM tactics such as genetically tolerant WHBs, essential oils, and organic acids, the use of synthetic miticides remains essential to the management of healthy WHB colonies (Delaplane et al. 2005). Therefore, it is imperative that new synthetic miticides with different chemistries be developed and registered for use in WHB colonies.

An important step in pesticide development is the laboratory bioassay. Formulations can be varied and tested in a dosage dependent manner, providing an efficient and controlled environment to evaluate the potential of pesticides to cause sufficient mortality of the target pest. Three bioassays have been used for testing miticides for *V. destructor*. Milani (1995) developed the wax disk method, in which miticide is incorporated into paraffin wax which is used to coat disks, to which individual *V. destructor* are exposed through contact. Elzen et al. (1999) adapted the glass vial method for *V. destructor*; it involves placing *V. destructor* within vials coated with a miticide. Recently, the complete exposure method has been developed. This bioassay allows for simultaneous determination of LC_{50}s for WHBs and *V. destructor* (Lindberg et al. 2000).

The objective of this study is to assess the activity of synthetic miticides against *V. destructor* using a glass vial bioassay. In the glass vial bioassay, *V. destructor* are exposed to tested miticides when they contact the walls of the glass vial which has been coated with a thin layer of the tested miticide. The

results will indicate which miticides and corresponding concentrations are feasible for further development as synthetic miticides within IPM programs for *V. destructor.*

2.2. Materials and Methods

Research was conducted at the Crop Diversification Center North, Alberta Agriculture and Rural Development, in Edmonton, Alberta, Canada.

2.2.1. Miticides

The synthetic miticides used in bioassays were commercially available products registered to control the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) through contact toxicity. From the range of synthetic miticides registered in Canada for *T. urticae*, miticides were eliminated that were similar in chemistry to pre-existing registered *V. destructor* miticides or had published high honey bee toxicity. The commercial synthetic miticides used in this bioassay were Apollo®, Floramite®, Forbid®, Shuttle®, and Vendex®; pertinent information regarding these miticides is summarized in Table 2.1.

2.2.2. Vial preparation

Miticide formulations were screened using a glass vial bioassay (Elzen et al. 1999; Kanga et al. 2010). Each commercial miticide formulation was serially diluted in acetone to create stock solutions of the following concentrations: 10, 5, 1, 0.5, 0.1, and 0.05% v/v or w/v. Acetone alone was used for the control

group (Elzen et al. 1999). The 10% stock solution for each miticide was made in a 125 mL Erlenmayer flask which was placed on a stirring plate for 10 minutes. 0.5 mL from each stock solution was pipetted into 20 mL borosilicate glass scintillation vials (61 mm x 28 mm, Cole-Palmer, Montreal, QC, Canada). Five replicate vials were made for each concentration; 10 replicate vials were made for the control group. The glass vials were then placed without caps on an industrial hot dog roller (Adcraft®, Hicksville, NY, USA) which rolled the vials to coat the inner surface of each vial. Vials were left on the hot dog roller until the acetone had completely evaporated (20 min – 3 hours depending on miticide). The lids were replaced once the vials were dry and were stored on the laboratory bench at 20°C and used within one month.

2.2.3. Varroa destructor collection

The sugar roll method used by Macedo et al. (2002) was adapted to obtain large numbers of *V. destructor* for use in the glass vial bioassay. A customized 19 L tote (Sterilite®, Townsend, MA, USA) was used to collect WHBs from colonies known to have significant *V. destructor* infestations. A large portion of the lid (20 cm X 26 cm) had been excised and replaced with an 8 mesh screen which was duct taped onto the lid. WHBs were shaken off brood frames into the tote until the tote was approximately 1/3 full. After the WHBs were in the tote, the lid was replaced and it was brought back to the laboratory. Icing sugar was sifted through the screen onto the WHBs (approximately 15 g/300

WHBs) and the box was rotated to evenly coat the WHBs with icing sugar. The box was then inverted and vigorously shaken onto a white surface. Phoretic *V. destructor* attached to the WHBs were dislodged and fell through the screen. Moving *V. destructor* were picked up with a small paintbrush (size 1-4) and kept in a Petri dish until they could be placed into vials. Any discolored or non-motile *V. destructor* were discarded.

2.2.4. Bioassays

To conduct the bioassay, 10 *V. destructor* were placed into each pre-prepared vial. One WHB pupa was placed in the vial with the *V. destructor.* The *V. destructor* within the vial were examined at 24 hours for mortality. Each *V. destructor* was prodded and counted as dead if it failed to elicit a leg kicking response.

A preliminary bioassay was performed to determine whether the control vials should be left empty or coated with acetone as recommended by Elzen et al. (1999). The *V. destructor* mortality at 24 hours of 10 vials coated with acetone was compared with 10 non-treated vials and subjected to a t-test. Following the results of this preliminary bioassay, all subsequent bioassays used acetone vials as controls.

Five replicate vials per miticide concentration and 10 replicate vials for the control group were used for each miticide bioassay. Therefore each miticide bioassay used 40 vials, and 400 *V. destructor*. The miticides bioassay was

replicated three separate times for each of the five miticides.

2.2.5. Statistical analysis

The LC_{50} of each miticide was estimated using Proc Probit in SAS (SAS Institute 2011) which fits the relationship between probability units of the mortality data and the logarithm of the concentration (Finney 1947). Proc Probit accounts for natural mortality using the Optc option in the model statement and uses a heterogeneity factor whenever the Pearson chi-squared test approaches significance ($p < 0.1$). All replicate trials for each miticide were analyzed separately and then the trials were pooled for each miticide to facilitate comparison among treatments. When 95% confidence limits could not be calculated due to heterogeneity in the probit model, 85% or 75% confidence limits were calculated (Cain et al. 1986). The differences between LC_{50}s were considered statistically significant if the ratio of the LC_{50} confidence limits failed to bracket 1.0 (Robertson and Preisler 1992).

2.3. Results

The t-test performed to assess the results of the preliminary bioassay showed that there was no significant difference in mite mortality between vials treated with acetone and non-treated vials ($t(18)=0.8182$, $P=0.4240$). Therefore vials treated with acetone were used as the control group for all miticides bioassays.

High mortality was observed for most miticides at 10% and then

mortality gradually decreased to the level of the control with successive dilutions in the miticide bioassays (Figure 2.1). The miticide LC_{50}s calculated through Probit analyses for the replicate trials of Apollo®, Floramite®, Forbid®, and Shuttle® ranged from 0.3% to 10% (Table 2.2). Vendex exhibited peculiar probit models with very shallow slopes for all replicate trials which led to the calculation of very large LC_{50}s and confidence limits.

Variability between replicate trials for each miticide was observed and is indicated by pairwise ratios between LC_{50} confidence limits (Table 2.3). When the replicates were pooled, pairwise ratios between LC_{50} confidence limits indicated the LC_{50} for Shuttle® was significantly lower than the other miticides (Figure 2.2, Table 2.4). Due to irregularity in the probit model for Vendex® (and resultant large confidence intervals), the pairwise ratios did not indicate significant differences between Vendex® and any other miticide even though the Vendex® LC_{50} is numerically higher (Figure 2.2, Table 2.4).

2.4. Discussion

This study is the first report on the effectiveness of five synthetic miticides registered for *T. urticae* against *V. destructor.* The tested miticides belong to different chemical groups than existing synthetic miticides for *V. destructor.* This study confirms that the glass vial bioassay is an effective method for testing miticides for their activity against *V. destructor* under laboratory conditions. The results suggest that Shuttle®, Forbid®, Apollo® and Floramite®

could be candidates for further testing as control agents for *V. destructor*. Vendex® did not exhibit effective miticidal activity and is unlikely to be useful in management of *V. destructor.*

2.4.1. Comparisons with Phytoseiidae

Although it is impossible to compare the results of this study with other research regarding the tested miticides and *V. destructor,* studies have frequently examined the effect of synthetic miticides on several species of predatory mites belonging to the Phytoseiidae family (Acari: Phytoseiidae). Like *V. destructor,* phytoseiid mites belong to the Acarine order Mesostigmata. Therefore, it may be prudent to consider literature regarding the effect of five tested miticides on phytoseiid mites.

Our study shows that Forbid® causes *V. destructor* mortality (Figure 2.1). Spiromesifen (active ingredient of Forbid®) was shown to be slightly harmful to a phytoseiid mite species (Kaplan et al. 2012) and decreased the life span of another species (Irigaray and Zalom 2006). An additional study showed further toxicity of spiromesifen to other phytoseiids (Cloyd et al. 2006). Furthermore, Irigaray and Zalom (2006) demonstrated that spiromesifen greatly decreased the fertility of the phytoseiid mite, *Galendromus occidentalis* Nesbitt. If spiromesifen causes decreased fertility of *V. destructor* as it does *G. occidentalis,* it would be invaluable as a synthetic miticide for *V. destructor*.

Vendex® was not toxic to *V. destructor* in this bioassay. This finding is

similar to other studies which have shown limited toxicity of its active ingredient, fenbutatin oxide, to phytoseiid mites (Kim and Yoo 2002; Qerhaili and Halloum 2012). However, azocyclotin, which is in the same chemical group as fenbutatin oxide, is highly toxicity to phytoseiid mites (Kim and Seo 2001; Qerhaili and Halloum 2012). Therefore, future research could determine if azocyclotin is effective against *V. destructor.*

2.4.2. Bioassay recommendations

A useful inclusion in the bioassay would have been another synthetic miticide known to be effective within WHB colonies. Apivar® is a synthetic miticide formulated in plastic strips that contains the active ingredient amitraz; it is currently very effective in managing *V. destructor* in Alberta (Nasr et al. 2010). If access to a commercial formulation of amitraz was available for the study, it would have provided a valuable comparison.

Large heterogeneity was observed within most of the probit models and could be due to a variety of reasons. Robertson and Preisler (1992) recommend minimizing model variability by using similar pest populations in biossays. The *V. destructor* used in the glass vial bioassay were sourced from several colonies over the course of four months, and therefore could be variable genetically and in terms of age structure. At present, bioassays for *V. destructor* are very labor intensive, limiting the number that can be performed within a day. If more bioassays could be performed in a day, a less variable *V. destructor* population

would be used, and the variability among replicate bioassays would likely decrease.

Presently, infested colonies of WHB and *V. destructor* must be kept nearby in order to perform bioassays, a situation which is expensive, unreliable, and time-consuming. *In vitro* propagation of pests for bioassays greatly increases the potential of successful product development but researchers have thus far been unable to develop a method of propagating *V. destructor* with an *in vitro* approach (Dietemann et al. 2012). If a method for *in vitro* rearing of *V. destructor* could be developed, it would undoubtedly increase efficiency of *V. destructor* bioassays and decrease the variability in the bioassayed *V. destructor* population.

2.4.3. Further development

Demonstration of miticidal activity on *V. destructor* is a crucial first step in miticide development. The results of this study warrant further inquiries into colony effects, efficacy, residues, and delivery systems for these miticides. While substantial further research is required before these miticides can be used by beekeepers, these miticides have potential to function as future synthetic miticides for IPM of *V. destructor.*

Tables

Table 2.1. Commercial formulations of miticides used in the glass vial bioassay.

Miticide	Active Ingredient	Group	Mode of Action	Honeybee Toxicity	Manufacturer
Apollo®	50% clofentezine	10	Mite growth inhibitors with unknown or non-specific action	Not acutely toxic	MANA Crop Protection
Floramite®	22.6% bifenazate	35	Neuronal inhibitors with unknown mode of action	Moderately toxic	OHP
Forbid®	45.2% spiromesifen	23	Inhibitors of lipid synthesis	Not acutely toxic	Bayer CropScience
Shuttle®	15.8% acequinocyl	20	Coupling site II electron transport inhibitors	Not acutely toxic	Arysta LifeScience
Vendex®	50% fenbutatin oxide	13	Inhibitors of oxidative phosphorylation, disruption of ATP formation	Not acutely toxic	DuPont

Table 2.2. Responses of *V. destructor* to tested miticides in each of three trials, and all three trials pooled, using the glass vial bioassay method. The table also summarizes the slopes, LC_{50} and *P* values for each trial and pooled trials.

Miticide	Trial	Number of Replicates	Slope ± SE	LC_{50} (95% CL)	χ^2	*P*
Apollo®	Trial 1	40	1.25 ± 0.63	9.391 (85% CL (3.251-9.505E+03)	3.94	0.0471
	Trial 2	40	3.21 ± 0.51	2.959(2.113-3.756)	40.25	<.0001
	Trial 3	36	2.70 ± 0.84	3.398 (0.129-7.423)	10.22	0.0014
	Trials Pooled	116	2.38 ± 0.30	4.268(3.469-5.022)	63.39	<.0001
Floramite®	Trial 1	40	2.67 ± 1.07	8.057 (5.262-14.605) **a**	6.22	0.0127
	Trial 2	40	0.76 ± 0.22	10.182 (4.632-49.960) **ab**	11.88	0.0006
	Trial 3	40	4.58 ± 1.14	5.111 (3.754-6.013) **b**	16.15	<.0001
	Trials Pooled	120	1.99 ± 0.37	6.267 (4.949-7.705)	28.23	<.0001
Forbid®	Trial 1	40	1.36 ± 0.56	7.700 (85% CL (3.325-66.583)	5.97	0.0146
	Trial 2	40	2.62 ± 0.44	4.065 (3.002-5.130)	35.15	<.0001
	Trial 3	36	1.54 ± 0.29	3.737 (2.372-5.582)	28.19	<.0001
	Trials Pooled	116	1.73 ± 0.30	4.630 (2.699-7.904)	33.62	<.0001
Shuttle®	Trial 1	34	0.83 ± 0.32	0.330 (85% CL (0.014-1.561) **a**	6.66	0.0099
	Trial 2	40	1.88 ± 0.32	2.500 (1.564-3.573) **b**	34.2	<.0001
	Trial 3	40	0.75 ± 0.13	3.299 (1.795-6.997) **b**	33.48	<.0001
	Trials Pooled	114	0.87 ± 0.08	1.341 (0.936-1.877)	116.78	<.0001
Vendex®	Trial 1	35	0.48 ± 0.21	2.45 (85% CL (0.279-1.717E+02)	5.18	0.0228
	Trial 2	40	0.12 ± 0.10	1.297E+04 (75% CL (84.328 - 1.996E262)	1.37	0.2424
	Trial 3	40	-0.14 ± 0.12	7.798E-06 (75% CL (2.141E-249 - 0.002)	1.38	0.2408
	Trials Pooled	115	0.17 ± 0.09	4.556 E+02 (85% CL (13.503-1.719E172)	3.26	0.0709

LC_{50} values were calculated using Probit analyses accounting for natural mortality in controls. All concentrations are expressed as % v/v with the exception of Vendex® which is % w/v. Confidence limits are 95% except when 85% or 75% confidence limits are indicated. Each replicate consists of a vial containing 10 *V. destructor.* Miticide trials were not significantly different except when followed by different letters according to the method of Robertson and Preisler (1992).

Table 2.3. Ratios between the LC_{50}s for replicated trials of each miticide.

Miticide	Comparison:	Ratio (95% Confidence Limits)
Apollo®	Trial 2 vs Trial 1	0.315 (0.079-1.257)
	Trial 2 vs Trial 3	0.871 (0.462-1.642)
	Trial 3 vs Trial 1	0.362(0.083-1.576)
Floramite®	Trial 1 vs Trial 2	0.791 (0.294-2.133)
	Trial 3 vs Trial 2	0.502 (0.192-1.312)
	Trial 3 vs Trial 1	0.634 (0.436-0.923)*****
Forbid®	Trial 2 vs Trial 1	0.528 (0.181-1.538)
	Trial 3 vs Trial 2	0.919 (0.570-1.484)
	Trial 3 vs Trial 1	0.485 (0.159-1.479)
Shuttle®	Trial 1 vs Trial 2	0.132 (0.021-0.844)*****
	Trial 2 vs Trial 3	0.758(0.355-1.620)
	Trial 1 vs Trial 3	0.100 (0.015-0.687)*****
Vendex®	Trial 1 vs Trial 2	1.88E-04 (7.18E-12 - 4.930E+03)
	Trial 3 vs Trial 2	6.002E-10 (4.155E-21 - 86.735)
	Trial 3 vs Trial 1	3.191E-06 (1.16E-14 - 8.780E+02)

Ratios with 95% confidence limits that fail to bracket 1.0 are considered to be significant according to the rule of Robertson and Preisler (1992). An asterisk (*) marks those ratios that fail to include 1.0.

Table 2.4. Ratios between the LC_{50}s of tested miticides.

Miticides Compared	Ratio (95% Confidence Limit)
Apollo vs Floramite	1.468 (1.113-1.937)*****
Apollo vs Forbid	1.085 (0.738-1.594)
Apollo vs Vendex	1.066E+02 (0.070-1.628E+05)
Floramite vs Vendex	72.627 (0.048-1.109E+05)
Forbid vs Floramite	1.354 (0.908-2.017)
Forbid vs Vendex	98.301(0.064-1.509E+05)
Shuttle vs Apollo®	0.314 (0.212-0.466)*****
Shuttle vs Floramite	0.214(0.142-0.321)*****
Shuttle vs Forbid	0.290 (0.178-0.471)*****
Shuttle vs Vendex	3.394E+02 (0.221-5.212E+05)

Replicate trials were pooled prior to pairwise comparison between miticides. Ratios with 95% confidence limits that fail to bracket 1.0 are considered to be significant according to the rule of Robertson and Preisler (1992). An asterisk (*) marks those ratios that do not bracket 1.0.

Figures

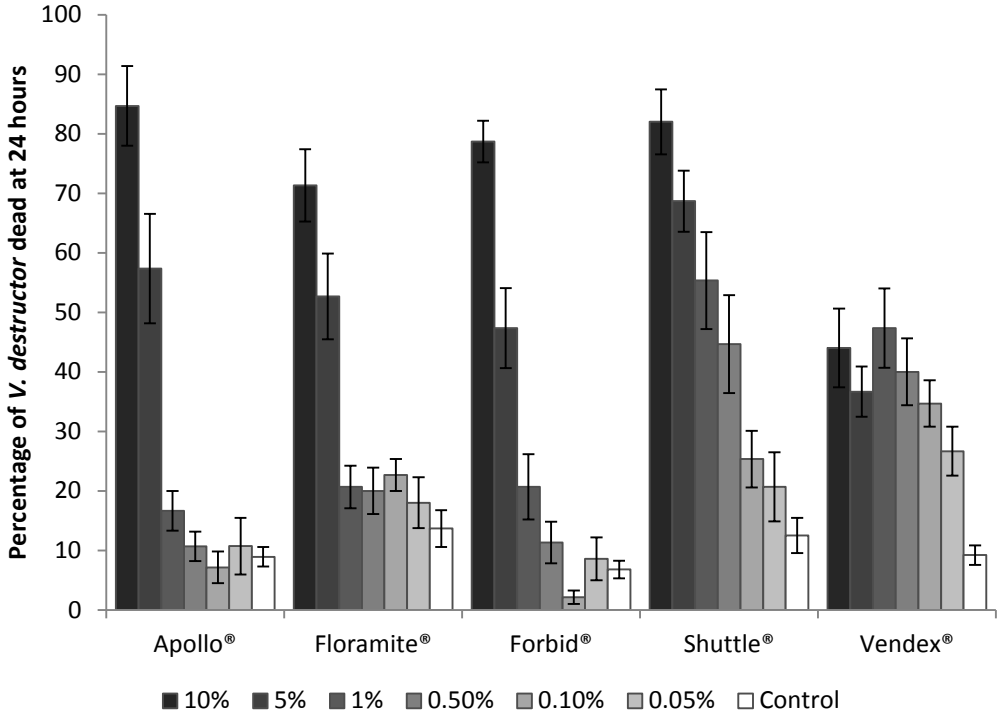

Figure 2.1. Mean proportion ± SE of *V. destructor* dead at concentrations (shown from high to low) used in the glass vial bioassay with respective control mortality for various tested miticides. Replicates for each miticide were pooled.

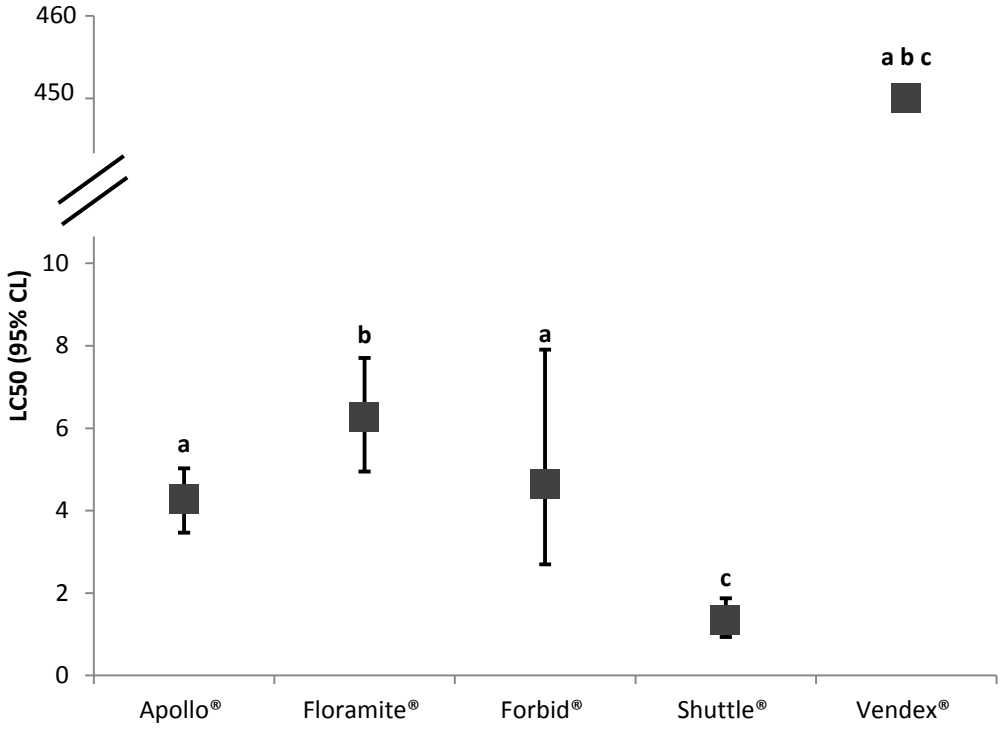

Figure 2.2. Estimated LC_{50} values from the glass vial bioassay for five miticides. Bars indicate the 95% confidence limits for each miticide (except for Vendex® which could not be calculated). Miticides followed by the same letter are not significantly different according to the method of Robertson and Preisler (1992) (Table 2.4).

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3. Chapter Three: Efficacy of miticides for Integrated Pest Management of *Varroa destructor* (Acari: Varroidae) under Canadian prairie conditions.

3.1. Introduction

Colonies of the Western Honey Bee (WHB), *Apis mellifera* L. (Hymenoptera: Apidae), are susceptible to numerous health threats. Perhaps no threat is more damaging at the colony level than the ectoparasitic mite *Varroa destructor* Anderson & Trueman 2000 (Acari: Varroidae). *Varroa destructor* feeds on the hemolymph of pupae and adult WHBs, causing numerous physiological symptoms, and vectoring harmful viruses such as Deformed Wing Virus (Dainat et al. 2012). Without management intervention, *V. destructor* infestation will cause colony mortality and significant economic losses for beekeepers involved in pollination and honey production (Currie et al. 2010).

Integrated pest management (IPM) for *V. destructor* has become an integral part of beekeeping. It involves the use of monitoring methods, economic thresholds, and tactics to manage *V. destructor* populations (Delaplane et al. 2005). IPM tactics include the use of genetically tolerant WHBs, and miticides such as essential oils, organic acids, and synthetic miticides (reviewed in Rosenkranz et al. 2010). In Alberta, there are two treatment windows during which colonies can be treated: April-June before the honey production season, and September-November before the onset of winter. Beekeepers commonly use a synthetic miticide in one of these treatment windows and a miticide such

as an essential oil or organic acid in the opposite window as needed (Nasr et al. 2010). The use of a non-synthetic miticide in the opposite window facilitates the use of synthetic miticides only once a year, thereby managing resistance to synthetic miticides and reducing residues in the colony environment. Therefore, treatment options that can be rotated with synthetic miticides while providing a reasonable level of *V. destructor* mortality are valued by Albertan beekeepers.

Treatments incorporating the essential oil thymol are known to cause significant *V. destructor* mortality (reviewed by Imdorf et. al. 1999). A great range of efficacies and side-effects have been reported for thymol treatments, likely due to temperature requirements for evaporation and a narrow buffer zone between the thymol exposure necessary to cause adequate *V. destructor* mortality, and the amount that will cause WHB side-effects (Rosenkranz et al. 2010). Thymovar® (Pronatex Inc, Richmond, QC, Canada) is concentrated in cellulose wafers for prolonged activity; it is a unique delivery system for thymol oils, which have otherwise been applied in gels, vermiculite blocks and dusts (reviewed by Imdorf et al. 1999).

Hops (*Humulus lupulus* L.) extracts have recently come of interest as a potential miticide for *V. destructor* (DeGrandi-Hoffman et al. 2012). Hops extract contains a variety of organic acids that have previously been shown to have activity against other mite species (Jones et al. 1996). HopGuard™ (BetaTec Hop Products, Washington, DC, USA) is a miticide containing hop acids formulated in cardboard strips to be applied to WHB colonies.

Two miticides that are widely used within Alberta for management of *V. destructor* by beekeepers are Apivar® (Véto-pharma, Villebon-sur-Yvette, France) and formic acid (Nasr et al. 2010). Apivar® is a synthetic miticide containing the formamidine amitraz. Formic acid is an organic acid that is applied using various methods to WHB colonies.

The main objective of this research was to assess two additional miticides, Thymovar® and HopGuard™, within the *V. destructor* IPM systems of Alberta. To assess this, it was imperative to determine: 1) possible side effects on WHB colonies, 2) efficacy against *V. destructor*, 3) how they compare to currently used industry management standards (Apivar® and formic acid) and 4) effectiveness in both fall and spring treatment windows.

3.2. Materials and Methods

All trials were conducted at the Crop Diversification Center North, Alberta Agriculture and Rural Development, in Edmonton, Alberta, Canada. Trials were conducted during the fall treatment window of 2011 and the spring treatment window of 2012. Each trial period was divided into three stages: "Pre-treatment" which included colony strength assessments and *V. destructor* population assessments prior to placement into treatment groups; "Treatment", which included treatment application, colony strength assessments, and *V. destructor* population assessment; and "Finishing Treatment" which included the use of a

finishing treatment to evaluate efficacy through *V. destructor* population assessment.

3.2.1 WHB colonies

Colonies were housed in double chambered Langstroth hives situated on Apinovar screened bottom boards with a removable tray (Chapleau 2003). Overwintered colonies headed with Hawaiian queens were used. Sugar syrup in division board feeders was fed to all colonies when required as part of routine management; the syrup contained Fumagilin-B (MediVet Pharmaceuticals, High River, AB, Canada) as recommended for nosemosis treatment (Nasr et al. 2008). Colonies were free from American foulbrood during the trial, and samples were taken periodically to determine *Nosema* spp. infection.

3.2.2. Colony strength assessments

Colony strength assessments were performed to estimate the area of capped brood, the area of frames covered with WHBs, and the amount of stored honey for each colony. The area of one side of a standard langstroth comb was measured to be 900 cm^2; therefore the area of brood, WHBs, and honey was recorded by visually estimating what proportion of the entire comb was covered by each to the nearest 30 cm^2 (after Skinner et al. 2001). All combs in the colony were removed and both sides were estimated visually.

3.2.3. Varroa destructor population assessments

Two methods were used to assess the *V. destructor* population during the trials. The number of phoretic *V. destructor* on a sample of adult WHBs was quantified using the alcohol wash method and expressed as *V. destructor* infestation. Secondly, the natural *V. destructor* mortality within colonies was quantified using sticky traps and expressed as *V. destructor* mortality per day.

The alcohol wash method was used to assess *V. destructor* infestation (De Jong et al. 1982). Samples of approximately 300 WHBs were removed from brood combs and preserved in 70% ethanol. The samples were agitated on a 175 rpm orbital shaker for 15 minutes and then the sample was rinsed repeatedly with running water using a 12 mesh strainer. Individual *V. destructor* fell through the strainer and were subsequently collected in an 11 Litre Rubbermaid® basin and counted. The WHBs left in the strainer were weighed to determine the number of WHBs in the sample. The number of WHBs in each sample was calculated based on the average weight of a single WHB as determined by weighing three samples of 10 WHBs per sampling date. The number of *V. destructor* found in the sample was then divided by the number of WHBs in the sample to determine the *V. destructor* infestation.

Varroa destructor mortality within each colony was monitored using sticky traps (Contech Enterprises Inc., Victoria, BC, Canada) placed on the tray beneath the Apinovar screened bottom board (Chapleau 2003). Dying *V. destructor* within the colony fell through the screen onto the sticky traps. Sticky

traps were periodically removed and were covered with plastic wrap; the number of adult *V. destructor* on the sticky trap was then counted. *V. destructor* mortality per day was calculated by dividing the number of *V. destructor* found on the sticky trap by the number of days the sticky trap was in place.

3.2.4. Fall 2011 Trial

3.2.4.1. Pre-treatment

All colonies were checked to ensure queen presence prior to the onset of the trial. The pre-treatment colony assessments were performed from 29 August – 1 September; samples of approximately 300 WHBs to determine *V. destructor* infestation were also taken during this time. *V. destructor* mortality per day was monitored pre-treatment using sticky traps for 5-7 days to establish a natural mortality baseline. Sticky traps were placed in the Apinovar tray on 29, 31 August, and removed 5 September.

3.2.4.2. Treatment

A complete randomized block experimental design was used when assigning treatments to account for variation in colony strength; colonies were blocked by brood area as determined by the pre-treatment colony assessment. Each treatment group consisted of eight colonies for a total of 40 colonies at the beginning of the trial. All treatments were placed on 5 September; remaining treatments were removed on 17, and 19 October. The five treatments were

Apivar®, Thymovar®, Hopguard™, formic acid, and a control. Apivar® strips were applied once according to recommendations of one strip for every four to five frames of WHBs in each brood chamber; the strips were left in for six weeks. Two wafers of Thymovar® were applied once to the top brood chamber and left in for six weeks. One HopGuard™ strip was applied for every four to five frames of WHBs in each brood chamber; the strips were left in for six weeks. Two 40 mL Dri-Loc® pads (Sealed Air Corp., Elmwood Park, NJ, USA) saturated with 65% formic acid were applied weekly for three weeks (5, 12, and 19 September) to the rear frame rests of the top brood chamber (Nasr et al. 2008). The control group received no treatment. Colony strength assessments were performed two weeks after treatment from 19-23 September, and six weeks after treatment from 17-19 October; samples of approximately 300 WHBs to quantify *V. destructor* infestation were also taken during these assessments. *V. destructor* population assessment also included sticky traps which were replaced regularly after treatment to determine *V. destructor* mortality (5, 8, 12, 16, 19, 23, 30 September; 7 October, removed 17 October).

3.2.4.3. Finishing treatment

In preparation for winter, colonies were wrapped with insulated winter wraps immediately after the treatment stage ended on 18 October and remained wrapped during the finishing treatment. Oxalic acid was applied as a finishing treatment to all treated colonies in order to quantify the number of *V.*

destructor remaining in the colonies for calculation of treatment efficacy relative to oxalic acid. Oxalic acid was applied as fumes using the Mitexx machine developed by Nasr et al. (2008). The applicator tip was inserted into the hive entrance and lengths of burlap were placed in the hive entrance to prevent fume loss. Two grams of oxalic acid applied as oxalic acid tablets (0.5 g each) were placed into the machine where they sublimated (Nasr et al. 2008). The oxalic applicator was removed after approximately 60-90 seconds when fumes started to emit from the top entrance of the wrapped colonies. Oxalic acid was applied to all colonies on 14 November and 28 November. Sticky traps were placed prior to oxalic acid application on 14 November, and replaced on 18 November, 28 November, and removed 5 December to assess *V. destructor* mortality.

3.2.5. Spring 2012 Trial

Methods used for the spring 2012 trial were the same as the fall 2011 trial except where indicated.

3.2.5.1. Pre-treatment

Colony strength assessments were performed from May 1-4, 2012; samples of approximately 300 WHBs to determine *V. destructor* infestation were also taken during this time. *V. destructor* mortality per day was monitored pre-treatment using sticky traps for 3 days (placed 4 May, removed 7 May) to establish a natural mortality baseline.

3.2.5.2. Treatment

Each treatment group consisted of 8 colonies with the exception of the control group which had 9 colonies. All treatments were placed on 7 May. There was no change to application of Apivar® or formic acid (reapplied 14 and 24 May). Treatments were removed 18-20 June. The number of applications for Thymovar® and HopGuard™ was changed from the fall 2011 trial. Thymovar® wafers were applied two times: the first application was on 7 May and wafers were replaced on 24 May according to label directions. The Hopguard™ strips were applied three times on 7, 14 and 24 May and removed on 31 May.

Colony strength assessments were performed three weeks after treatment on 28-29 May, and six weeks after treatment on 18-20 June; samples of approximately 300 WHBs to assess *V. destructor* infestation were collected during these assessments. *V. destructor* population assessment also included sticky traps which were replaced every 3-4 days after treatment to quantify *V. destructor* mortality (placed 7 May, replaced 10, 14, 17, 24, 28, 31 May, and 4, 7, 11, 14 June, removed 18 June).

3.2.5.3. Finishing treatment

Apivar® was used as a finishing treatment for the spring 2012 trial to quantify the number of *V. destructor* remaining in the colonies for calculation of treatment efficacy relative to Apivar®. After experimental treatments were removed on 18-20 June, one strip of Apivar® was placed for every four to five

frames covered with WHBs in each brood chamber and the strips were left in the colonies for six weeks until their removal on 30-31 July. Samples of approximately 300 WHBs were taken 9-10 July and 30-31 July to determine *V. destructor* infestation. Sticky traps were replaced every 7 days (placed 18 June, replaced 25 June, 3, 9, 16, 24 July, and removed 30 July) to assess *V. destructor* mortality during the finishing treatment stage.

3.2.6. Statistical analysis

All analyses were performed using SAS 9.3 for Windows (SAS Institute 2011). Data from colony strength assessments (area covered with WHBs, capped brood, and stored honey per colony) were square root transformed prior to analyses. *V. destructor* infestation data were arcsine-square root transformed prior to analyses and *V. destructor* mortality data were square root transformed.

The area of bees, brood, and honey was summed separately for the top brood chamber, bottom brood chamber and both brood chambers before being compared for each sampling date using one-way analysis of variance with Tukey means separation test.

The pre-treatment readings for *V. destructor* infestation and *V. destructor* mortality were analyzed with one-way analysis of variance with Tukey means separation to determine if significant differences existed before treatments were applied. The effect of treatment on *V. destructor* population assessments (*V. destructor* infestation and *V. destructor* mortality) was analyzed with repeated

measures analysis of variance with treatment modeled as a fixed effect and the pre-treatment readings used as a covariate (PROC MIXED, SAS Institute 2011).

Efficacy was calculated according to the following formula and arcsine transformed prior to analyses. Efficacy was subjected to one-way analysis of variance with Tukey means separation to determine if significant differences among treatments.

$$Efficacy = \frac{(\#of\ V.destructor\ killed\ by treatment)}{(\#of\ V.destructor\ killed\ by treatment) + (\#of\ V.destructor\ killed\ by\ finishing\ treatment)} x100$$

Colonies that lost queens during the experiment were removed from post-treatment data. Additionally, one outlier colony was removed from the fall 2011 experiment because it had a *V. destructor* infestation that was consistently 4-10 standard deviations higher than the average. Therefore, in fall 2011, the number of colonies per group pre-treatment was: Thymovar®, 7, and all other groups, 8. The number of colonies per group post-treatment in fall 2011 was: Apivar®, 7, formic acid, 5, HopGuard™, 8, Thymovar®, 6, and control, 8. In spring 2012, the number of colonies pre-treatment was: control, 9, and other groups, 8. Due to queen loss, the number of colonies post-treatment in spring 2012 was: Apivar®, 8, HopGuard™, 7, formic acid, 5, Thymovar®, 6, and control, 9.

3.3. Results

3.3.1. Fall 2011 Trial

3.3.1.1. Pre-treatment

The average results for the fall 2011 colony assessments are summarized in Table 3.1a and Table 3.1b. The average total area covered with brood per colony pre-treatment was 2163 ± 246 cm^2; WHB area was 4770 ± 456 cm^2; and honey was 11158 ± 959 cm^2. There was not a significant difference in total colony strength assessment parameters among treatments during the pre-treatment stage (Table 3.1b).

The overall average *V. destructor* infestation prior to treatment was 3.77 ± 0.62% (Figure 3.1). The pre-treatment *V. destructor* infestation in the Thymovar® group was significantly higher than the other groups (F=3.63, df=4, 33, P=0.0161, Tukey).

Average *V. destructor* mortality on sticky traps pre-treatment was 21.68 ± 4.38 *V. destructor*/day. Treatment means did not vary significantly prior to treatment (F=0.90, df=4, 33, P=0.4743).

3.3.1.2. Treatment

The average results for the fall 2011 colony strength assessments are summarized in Table 3.1a and Table 3.1b. The total area of brood six-weeks post-treatment was significantly higher in the formic acid treatment group than the Apivar® group (F=3.47, df=4, 29, P=0.0195, Tukey).

Repeated measures analysis showed a significant effect of treatment over time on *V. destructor* infestation (F=15.55, df=4, 28, P<0.0001) with significantly lower infestation observed over the course of the treatment stage (5 September – 18 October) in the Apivar®, formic acid, and Thymovar® groups than the control or HopGuard™ colonies (Figure 3.1). The percentage of infestation of the HopGuard™ group was not significantly different from the control (Figure 3.1).

Repeated measures analysis showed that *V. destructor* mortality per day was affected by treatment (F=4.18, df=4, 28, P=0.0089) with significantly more *V. destructor* mortality during the treatment stage (5 September – 18 October) in the Apivar® and Thymovar® colonies than in the HopGuard™ colonies (Figure 3.2). *V. destructor* mortality per day in the formic acid and HopGuard™ colonies was not statistically different from the control colonies (Figure 3.2).

3.3.1.3. Finishing treatment

There was an effect of treatment on efficacy as determined with the use of oxalic acid sublimation as a finishing treatment (F=14.60, df=4,33, P< 0.0001). The overall efficacy of treatment is summarized in Table 3.2. The efficacies of Apivar® (87.07 ± 2.69%), formic acid (78.48 ± 8.47%), and Thymovar® (88.91 ± 8.47%) were significantly higher than that of HopGuard™ (42.96 ± 6.46%) or the control group (28.69 ± 7.33%).

3.3.2. Spring 2012 Trial

3.3.2.1. Pre-Treatment

The average results for the spring 2012 colony assessments are summarized in Table 3.3a and Table 3.3b. The average total area covered with brood per colony pre-treatment was 2560 ± 143 cm^2; mean WHB area was 5343 ± 372 cm^2 and average honey area was 5731 ± 551 cm^2. There were no significant differences found among the colony strength assessments for the pre-treatment colony analysis (Table 3.3a, Table 3.3b).

The overall average *V. destructor* infestation pre-treatment was 4.49 ± 0.57% (Figure 3.4). Pre-treatment infestation means did not vary significantly by treatment (F=0.92, df=4, 35, *P*=0.4640). The average *V. destructor* mortality pre-treatment was 33.16 ± 3.63 *V. destructor*/day (Figure 3.5) There were no statistically significant differences among treatment means (F=0.66, df=4, 35, P=0.6236).

3.3.2.2. Treatment

Colony strength assessments at three weeks

The average results for the spring 2011 colony strength assessments are summarized in Table 3.3a and Table 3.3b. At three weeks post-treatment, the area of brood (total of both chambers) varied significantly among treatment group means (*F*=5.99, df=4, 30, *P*=0.0011). At this time, the average amount of brood in the Thymovar® colonies was 52 ± 14% less than the control (Table 3.4).

At three weeks post-treatment, the area of brood in the top chamber was significantly differently among treatments (F=19.67, df=4, 30, P<0.0001). The Thymovar® group had significantly less brood in the top chamber than the other treatment groups (Figure 3.3), which amounted to on average, 86 ± 7% less brood than the control (Table 3.4). Additionally, the amount of honey three weeks post-treatment in the top chamber varied significantly among treatment means (F=3.43, df=4, 30, P=0.0201), with significantly less honey in the formic acid treatment group than in the Apivar® or HopGuard™ groups (Table 3.3a).

The area of brood in the bottom chamber was significantly different among treatment means three weeks post-treatment (F=3.04, df=4, 30, P=0.0322) with significantly less brood in the formic acid treatment group than the Apivar® or HopGuard™ groups (Table 3.3a).

Colony strength assessments at six weeks

The total brood area varied significantly among treatment means (F=2.71, df= 4, 30, P=0.0487) six weeks post-treatment with significantly less brood in the Thymovar® group than the HopGuard™ group (Table 3.3b). Total honey area also varied significantly at six weeks (F= 3.84, df=4, 30, P=0.0123) with significantly less honey in the Thymovar® group than the control (Table 3.3b).

The brood area in the top chamber was significantly different among treatments six weeks post-treatment (F=7.59, df=4,30, P=0.0002) with significantly less brood in the Thymovar group than the formic acid group (Figure

3.3). The brood area in the top chamber for the Thymovar® colonies averaged 77 ± 13% less than the control colonies during this time (Table 3.4).

At six weeks post-treatment, bottom chamber WHB area was significantly different among treatment means (F=3.51, df=4, 30, P=0.0182) with significantly less WHB area in the formic acid group than in the HopGuard™ or control groups (Table 3.3a). The honey area in the bottom chamber also varied significantly six weeks post-treatment (F=3.71, df=4, 30, P=0.0144) with significantly less honey in the Thymovar® group than the control group (Table 3.3a).

Varroa destructor population assessments

Repeated measures analysis showed that treatment also affected the percentage of adult bees infested with *V. destructor* (F=18.4, df=4, 30, P<0.0001) over the course of the treatment stage (7 May – 18 June) when all treatment groups were significantly less infested than the control. The Apivar® and Thymovar® groups were also significantly less infested over time than the HopGuard™ group (Figure 3.4).

Repeated measures analysis also showed that treatment affected *V. destructor* mortality on sticky traps over time (F=8.05, df=4, 30, P=0.0002), with significantly more *V. destructor* mortality in the Apivar® group throughout the treatment stage (7 May – 18 June) than in all the other treatment groups; there was also significantly more *V. destructor* mortality over time in the Thymovar® group than the HopGuard™ group (Figure 3.5).

3.3.2.3. Finishing treatment

There was an effect of treatment on efficacy as determined with a finishing treatment of Apivar® (F=35.28, df=4, 35, P<0.0001). Apivar® (74.93 ± 3.18%), formic acid (71.90 ± 6.52%), and Thymovar® (82.33 ± 3.32%) were significantly more efficacious than HopGuard™ (43.56 ± 3.18%) or the control (24.09 ± 3.89%) (Table 3.5).

3.4. Discussion

This is the first study on the use of two non-synthetic miticides, HopGuard™ and Thymovar®, within the conditions of Alberta. Through regular colony strength assessments and *V. destructor* population assessments, any side-effects of treatment as well as the efficacy of the treatment could be demonstrated. The evaluation of these miticides alongside industry-standard miticides in both spring and fall demonstrates how they might best work within existing IPM systems.

3.4.1. Fall 2011 Trial

Apivar® effectively caused *V. destructor* mortality during the six weeks of application in fall 2011. The efficacy of 87.07 ± 2.69% is higher than the efficacy of 83.8 ± 3.5% reported by Floris et al. (2001). It is likely that this estimation of efficacy for all treatments is lower than expected because the remaining *V. destructor* population was able to continue reproducing in the month between the treatment stage and application of the finishing treatment. The efficacy

observed for Apivar® is consistent with previously reported results (Nasr et al. 2010) and allows for effective comparison with other treatments.

While formic acid colonies contained, on average, significantly more brood than other treatment groups six weeks post-treatment, this effect can likely be attributed to the small sample size due to loss of queens in this group (three queens were lost). The loss of queens in the formic acid group may have been caused by temperature extremes observed during the fall 2011 trial. Wallner and Fries (2003) recommend a temperature range of 12-25°C for the application of formic acid which was not met in the fall 2011 trial. The daily maximum temperature exceeded 25°C for several days after the first application of formic acid, and the daily minimum temperature was always below 12°C for the duration of the trial (Figure 3.6). The analyses of *V. destructor* infestation rates and efficacy data both confirm that formic acid provided significantly better management of *V. destructor* than the control. The calculated efficacy of formic acid was 78.48 ± 8.47% which is within the range of efficacies mentioned for formic acid of 51-100% by Ostermann and Currie (2004).

In this study, HopGuard™ activity was limited to three days after application; the WHBs removed much of the cardboard strip during this time. This finding is consistent with other studies involving oils formulated in cardboard strips which were shredded by the WHBs within one week (Skinner et al. 2001). Although large initial mortality (Figure 3.2) was observed for HopGuard™, it was not sufficient to provide any *V. destructor* control statistically

different from the control colonies. This is the first study that involves the application of HopGuard™ to colonies with brood. The presence or absence of brood during treatment is an important consideration as a large proportion of the *V. destructor* in a colony with brood are located beneath capped brood and therefore unexposed to treatments (Martin 1998). As brood was present, the efficacy for HopGuard™ in the fall 2011 trial of this study was 42.96 ± 6.46% which is much lower than the efficacy of 93.5% reported for HopGuard™ applied to winter colonies with no brood (Rademacher and Harz 2011). DeGrandi-Hoffman et al. (2012) applied HopGuard™ to five-frame colonies with caged queens and found that the majority of *V. destructor* mortality occurred within two days of application and recommended reapplications in the presence of brood.

Application of Thymovar® was not associated with any negative effects as determined by the colony population assessments in fall 2011. Thymovar® was highly effective in fall 2011 as it performed in a statistically similar manner to Apivar® in terms of *V. destructor* infestation over time, *V. destructor* mortality over time, and overall efficacy. Studies involving Thymovar® report a range of efficacies from 72% - 97% (Table 3.6); our observed efficacy of 88.91 ± 8.47% is well within this range. The ambient temperature was well above the historical average during the first three weeks of the fall 2011 trial (Figure 3.6), which may have contributed to the high efficacy observed for Thymovar®.

3.4.2. Spring 2012 Trial

The efficacy reported for Apivar® in the spring 2012 trial is 74.93 ± 3.18% which is lower than was expected. However, the values reported for spring 2012 efficacy of treatments are likely an underestimation of the actual value. As the finishing treatment was applied for a full six weeks, there was substantial opportunity for *V. destructor* in nearby infested colonies to reinvade (Kraus and Page 1995; Gregorc and Planinc 2005) and cause elevated finishing treatment mortality.

The colony assessment parameters for formic acid-treated colonies were frequently significantly different from some of the other treatment groups, including less honey (top brood chamber) and less brood (bottom brood chamber) three weeks post-treatment, along with fewer WHBs (bottom brood chamber) and more brood (top brood chamber) six weeks post-treatment (Table 3.3a). As these findings were not significant for the total of both brood chambers and the same finding was not repeated for both post-treatment readings, it is difficult to assess whether this is an effect of treatment, or whether it is due to the small size of this group (3 queens were lost). Treatment with formic acid caused an average efficacy of 71.90 ± 6.52% which was significantly higher than the control and HopGuard™ groups and within the range mentioned by Ostermann and Currie (2004).

Due to an absence of any lasting activity in the fall 2011 trial, HopGuard™ was applied three times in the spring 2012 trial. No negative effects on bees

were apparent. Each HopGuard™ treatment caused a peak in *V. destructor* mortality followed by a subsequent depression (Figure 3.5); this is similar to the findings of fall 2011 and confirms that HopGuard™ activity is limited to a short time immediately following treatment. Despite three applications of HopGuard™ in spring 2012, the efficacy of HopGuard™ was only 42.96 ± 6.46%, which was not significantly different than the control colonies.

Treatment with Thymovar® caused a drastic reduction in overall brood rearing that was most evident in the top brood chamber during the spring 2012 trial (Table 3.4, Figure 3.3). The reduction in brood rearing was likely associated with temperature fluctuations following Thymovar® application. The daily maximum temperature following the first application of Thymovar® ranged widely from 10°C to 25°C during the first four days of application (Figure 3.7). Floris et al (2004) also found substantial brood reduction while using other thymol-based miticides and recommended that they not be used during times when colonies are population building. Removal of honey and brood directly below the Thymovar® wafer was reported by Baggio et al. (2004). As the intensity of brood rearing in spring is directly correlated with honey production in summer (Szabo and Lefkovitch 1989), the brood reduction seen in this study (51% reduction in total brood three weeks after treatment) would undoubtedly have a negative effect on summer colony performance and honey yields. The efficacy found for Thymovar® was still quite high, 82.33 ± 3.32%, within the range seen in other studies (Table 3.6) and statistically similar to Apivar® (Table

3.4). However, it should be noted that the reduced brood area (Table 3.4) in the Thymovar® colonies would have limited the reproductive potential of *V. destructor* in these colonies in comparison to colonies that maintained large areas of brood throughout the experiment.

3.4.3. Treatment recommendations

It is difficult to compare *V. destructor* infestation of adult WHBs between seasons because of colony population dynamics. For instance, the control *V. destructor* infestation rose rapidly in fall 2011 (Figure 3.1) but appeared to remain the same in spring 2012 (Figure 3.4). Martin (1998) advises that infestation rates naturally rise in the fall because the WHB population is declining, and the decline in the amount of brood in fall results in more *V. destructor* attached to adult WHBs. Meanwhile in the spring, increased brood rearing results in a larger WHB population and more *V. destructor* moving from adult WHBs to brood cells for reproduction. Therefore, it is advisable to take into account seasonal *V. destructor* population dynamics while interpreting infestation data.

Many of the findings of this study were similar in both seasons, confirming that the products Apivar®, formic acid, and HopGuard™ can be used in either treatment window. However, HopGuard™ does not cause enough mortality to be effective in its present formulation. While a cardboard strip formulation has potential in niche areas such as the treatment of packages of

bees (DeGrandi-Hoffman et al. 2012), or broodless colonies (Rademacher and Harz 2011), our study shows that it is limited in its application to large reproducing colonies. It is possible that repeated applications of HopGuard™ (likely at least six) could cause sufficient *V. destructor* mortality for the treatment to have a reasonable efficacy. However, such a demanding treatment schedule is unlikely to be favored by commercial beekeepers, and current label recommendations for HopGuard™ preclude more than three treatments a year. As our data show that HopGuard™ is capable of causing substantial initial *V. destructor* mortality, a delivery system that could deliver the hop beta acids over an extended period of time would be more effective as a colony miticide.

The reduction in brood rearing in response to the treatment of Thymovar® in the spring 2012 trial is concerning, and preclude its recommendation for the prairie spring treatment window or any time colony populations are building (Floris et al. 2004). However, there was no significant effect of Thymovar® on brood production in the fall 2011 trial. Therefore it is likely that Thymovar® can be used safely in the fall treatment window when temperature requirements are met as any effects on brood production would be minimal due to an overall decline in brood rearing and tendency for the WHB cluster to move to the bottom chamber during this time.

In summary, this study shows that two widely used miticides, formic acid, and Apivar®, remain effective management options for both treatment windows on the Canadian prairies. Thymovar® is recommended for use during the fall

season only, and the efficacy of HopGuard™ would likely increase with a change in delivery system. There is potential for these two new miticides to be valuable components of beekeepers' IPM systems on the Canadian prairies.

Tables

Table 3.1a. Average area (± SE) of WHBs, brood, and honey in the top brood chamber and bottom brood chamber for each treatment group throughout the fall 2011 trial.

		Pre-treatment			Two weeks post-treatment			Six weeks post-treatment		
Brood Chamber	Treatment	WHBs (cm^2)	Brood (cm^2)	Honey (cm^2)	WHBs (cm^2)	Brood (cm^2)	Honey (cm^2)	WHBs (cm^2)	Brood (cm^2)	Honey (cm^2)
Top Brood Chamber	Apivar®	2794 ± 745	887 ± 301	12319 ± 756	3475 ± 386	184 ± 134	11705 ± 806	2138 ± 564	115 ± 115	12894 ± 444
	Formic Acid	2746 ± 748	1117 ± 279	11657 ± 1014	4568 ± 778	90 ± 53	10710 ± 852	3252 ± 580	32 ± 32	11652 ± 534
	HopGuard™	2569 ± 600	891 ± 253	11007 ± 905	2915 ± 302	544 ± 163	10294 ± 753	2702 ± 533	185 ± 85	11879 ± 708
	Thymovar®	3051 ± 659	1009 ± 334	11880 ± 915	3247 ± 513	237 ± 163	9538 ± 464	3882 ± 552	140 ± 106	11226 ± 692
	Control	3032 ± 818	1274 ± 439	11573 ± 949	3440 ± 710	714 ± 222	10141 ± 505	2766 ± 490	374 ± 242	11621 ± 531
Bottom Brood Chamber	Apivar®	4960 ± 753	2710 ± 493	4605 ± 609 **ab**	7581 ± 1286	1415 ± 171	4221 ± 695	7433 ± 702	801 ± 257 **a**	4410 ± 681
	Formic Acid	4778 ± 778	2339 ± 608	4181 ± 494 **a**	8439 ± 975	1252 ± 341	3232 ± 902	9026 ± 889	2245 ± 353 **b**	3994 ± 792
	HopGuard™	4988 ± 854	2810 ± 385	7306 ± 591 **b**	5694 ± 691	1569 ± 285	6706 ± 660	5407 ± 623	1048 ± 95 **a**	7339 ± 791
	Thymovar®	4327 ± 932	2664 ± 503	5659 ± 956 **ab**	6640 ± 998	2065 ± 319	5075 ± 1128	6909 ± 1236	1005 ± 302 **a**	5059 ± 1193
	Control	5310 ± 1094	2274 ± 391	4883 ± 533 **ab**	5351 ± 725	1851 ± 431	4730 ± 705	7177 ± 883	794 ± 87 **a**	5528 ± 978

The number of colonies pre-treatment was: Thymovar® (7) and other groups: (8). Due to queen loss, the number of colonies post-treatment was: Apivar® (7), formic acid (5), HopGuard™ (8), Thymovar® (6), and control (8). Treatment differences among parameters are significant when followed by different letters ($p<0.05$). When no significant differences were found within a parameter, no letters were placed.

Table 3.1b. Average area (± SE) of WHBs, brood, and honey for the total of both brood chambers for each treatment group throughout the fall 2011 trial.

		Pre-treatment			Two weeks post-treatment			Six weeks post-treatment		
Brood Chamber	Treatment	WHBs (cm^2)	Brood (cm^2)	Honey (cm^2)	WHBs (cm^2)	Brood (cm^2)	Honey (cm^2)	WHBs (cm^2)	Brood (cm^2)	Honey (cm^2)
Total of Both Brood Chambers	Apivar®	7755 ± 1374	3597 ± 391	16923 ± 931	11055 ± 1582	1599 ± 202	15926 ± 1193	9571 ± 641	917 ± 234 **a**	17304 ± 915
	Formic Acid	7524 ± 1087	3456 ± 544	15839 ± 989	13006 ± 1344	1342 ± 313	13942 ± 1140	12277 ± 1244	2277 ± 350 **b**	15645 ± 1113
	HopGuard™	7556 ± 1188	3702 ± 290	18314 ± 290	8609 ± 830	2113 ± 284	17000 ± 1108	8109 ± 1088	1234 ± 122 **ab**	19218 ± 1327
	Thymovar®	7378 ± 1459	3673 ± 501	17539 ± 1627	9887 ± 1474	2301 ± 347	14613 ± 1425	10790 ± 1757	1145 ± 280 **ab**	16285 ± 1478
	Control	8343 ± 1682	3548 ± 489	16456 ± 1320	8790 ± 1263	2565 ± 490	14871 ± 1080	9944 ± 1265	1169 ± 249 **ab**	17149 ± 1412

The number of colonies pre-treatment was: Thymovar® (7) and other groups: (8). Due to queen loss, the number of colonies post-treatment was: Apivar® (7), formic acid (5), HopGuard™ (8), Thymovar® (6), and control (8). Treatment differences among parameters are significant when followed by different letters ($p<0.05$). When no significant differences were found within a parameter, no letters were placed.

Table 3.2. Average (± SE) cumulative *V. destructor* mortality on sticky traps in response to treatments and in response to the finishing treatment for the fall 2011 trial. The resultant average (± SE) efficacy of treatments relative to the finishing treatment is given.

Treatment	Treatment Mortality	Finishing Treatment Mortality	Efficacy (%)
Apivar®	5089.29 ± 868.65	780.29 ± 192.40	87.07 ± 2.69 **a**
Formic Acid	2371.40 ± 434.04	627.00 ± 195.08	78.48 ± 8.47 **a**
HopGuard™	1182.25 ± 350.27	2412.50 ± 734.45	42.96 ± 6.46 **b**
Thymovar®	6688.33 ± 1971.28	369.00 ± 453.92	88.91 ± 8.47 **a**
Control	652.38 ± 217.46	1762.13 ± 453.92	28.69 ± 7.33 **b**

Efficacies followed by different letters are significantly different according to one-way analysis of variance (Tukey, $p<0.05$). Due to queen loss, the number of colonies was: Apivar® (7), formic acid (5), HopGuard™ (8), Thymovar® (6), and control (8).

Table 3.3a. Average area (± SE) of WHBs, brood, and honey for the top brood chamber and bottom brood chamber for each treatment group throughout the spring 2012 trial.

		Pre-Treatment			Three weeks post-treatment			Six weeks post-treatment		
Brood Chamber	Treatment	WHBs (cm2)	Brood (cm2)	Honey (cm2)	WHBs (cm2)	Brood (cm2)	Honey (cm2)	WHBs (cm2)	Brood (cm2)	Honey (cm2)
Top Brood Chamber	Apivar®	3702 ± 369	2403 ± 301	3206 ± 757	4573 ± 452	3169 ± 372 **a**	3435 ± 670 **a**	2911 ± 231	1440 ± 459 **ac**	6220 ± 999
	Formic Acid	3988 ± 457	2117 ± 323	2714 ± 672	4723 ± 734	2955 ± 278 **a**	1245 ± 415 **b**	3858 ± 400	3684 ± 477 **b**	3774 ± 625
	HopGuard™	3710 ± 562	2327 ± 306	3290 ± 903	4129 ± 311	3124 ± 321 **a**	4074 ± 770 **a**	4088 ± 717	2198 ± 354 **bc**	5668 ± 909
	Thymovar®	3722 ± 453	2677 ± 410	2504 ± 565	3280 ± 421	500 ± 231 **b**	2376 ± 422 **ab**	3263 ± 571	473 ± 269 **a**	3296 ± 941
	Control	4462 ± 594	2222 ± 384	3000 ± 774	4366 ± 448	3498 ± 377 **a**	3106 ± 666 **ab**	4480 ± 661	2061 ± 350 **bc**	5796 ± 566
Bottom Brood Chamber	Apivar®	1887 ± 585	238 ± 178	2210 ± 553	6573 ± 950	2883 ± 551 **a**	1339 ± 482	6122 ± 808 **ab**	3222 ± 717	2379 ± 585 **ab**
	Formic Acid	540 ± 281	121 ± 108	3093 ± 865	3052 ± 1020	581 ± 297 **b**	1574 ± 546	3439 ± 710 **a**	1252 ± 469	994 ± 299 **ab**
	HopGuard™	2274 ± 813	480 ± 167	2605 ± 767	6194 ± 716	2479 ± 405 **a**	1585 ± 439	7544 ± 760 **b**	3092 ± 403	2336 ± 521 **ab**
	Thymovar®	843 ± 260	161 ± 140	2637 ± 578	6032 ± 516	2161 ± 545 **ab**	1430 ± 612	4661 ± 532 **ab**	2478 ± 531	941 ± 535 **a**
	Control	1509 ± 454	86 ± 45	3323 ± 794	5039 ± 966	2032 ± 479 **ab**	2018 ± 810	7409 ± 1014 **b**	2778 ± 633	3108 ± 478 **b**

The number of colonies pre-treatment was: control (9), and other groups (8). Due to queen loss, the number of colonies post-treatment was: Apivar® (8), HopGuard™ (7), Formic Acid (5), Thymovar® (6), and Control (9). Treatment differences among parameters are significant when followed by different letters (Tukey, $p<0.05$). When no significant differences were found within a parameter, no letters were placed.

Table 3.3b. Average area (± SE) of WHBs, brood, and honey for the top brood chamber, bottom brood chamber, and the total of both brood chambers for each treatment group throughout the spring 2012 trial.

		Pre-Treatment			Three weeks post-treatment			Six weeks post-treatment		
Brood Chamber	Treatment	WHBs (cm2)	Brood (cm2)	Honey (cm2)	WHBs (cm2)	Brood (cm2)	Honey (cm2)	WHBs (cm2)	Brood (cm2)	Honey (cm2)
Total of Both Brood Chambers	Apivar®	5589 ± 717	2641 ± 231	5415 ± 1148	11146 ± 1243	6052 ± 638 **a**	4774 ± 929	9033 ± 908	4661 ± 477 **ab**	8599 ± 1457 **ab**
	Formic Acid	4528 ± 628	2238 ± 333	5806 ± 1392	7774 ± 1700	3536 ± 516 **a c**	2819 ± 838	7297 ± 748	4935 ± 808 **ab**	4768 ± 561 **ab**
	HopGuard™	5984 ± 1209	2806 ± 324	5895 ± 1481	10323 ± 823	5604 ± 336 **a**	5659 ± 1079	11631 ± 1416	5290 ± 246 **a**	8005 ± 1370 **ab**
	Thymovar®	4565 ± 633	2839 ± 308	5141 ± 678	9312 ± 903	2661 ± 763 **bc**	3806 ± 558	7925 ± 664	2952 ± 689 **b**	4237 ± 1027 **a**
	Control	5971 ± 865	2308 ± 382	6323 ± 1494	9405 ± 1349	5530 ± 594 **a**	5124 ± 1395	11889 ± 1511	4839 ± 557 **ab**	8903 ± 930 **b**

The number of colonies pre-treatment was: control (9), and other groups (8). Due to queen loss, the number of colonies post-treatment was: Apivar® (8), HopGuard™ (7), Formic Acid (5), Thymovar® (6), and Control (9). Treatment differences among parameters are significant when followed by different letters (Tukey, $p<0.05$). When no significant differences were found within a parameter, no letters were placed.

Table 3.4. Average (±SE) percent reduction of brood area (cm^2) in Thymovar® treated colonies (*n* = 6) in comparison to control treated colonies (*n* = 9).

Time Since Treatment	Brood Area Reduction (%)	Top Chamber Brood Area Reduction (%)
Three Weeks	52 ± 14	86 ± 7
Six Weeks	39 ± 14	77 ± 13

Table 3.5. Average (±SE) cumulative *V. destructor* mortality on sticky traps in response to treatments and the finishing treatment for the spring 2011 trial. The resultant average (± SE) efficacy of treatments relative to the finishing treatment is given.

Treatment	Treatment Mortality	Finishing Treatment Mortality	Efficacy (%)
Apivar®	2821.13 ± 453.43	927.63 ± 201.30	74.93 ± 3.18 **a**
Formic Acid	1400.83 ± 214.70	531.50 ± 111.60	71.90 ± 6.52 **a**
HopGuard™	1847.43 ± 455.01	2399.57 ± 578.80	43.56 ± 3.18 **b**
Thymovar®	2351.50 ± 328.72	475.83 ± 92.97	82.33 ± 3.32 **a**
Control	1008.67 ± 236.08	2907.78 ± 395.26	24.09 ±3.89 **b**

Efficacy values followed by different letters indicate significant difference according to one-way analysis of variance (Tukey, $p<0.05$). Due to queen loss, the number of colonies was: Apivar® (8), HopGuard™ (7), Formic Acid (5), Thymovar® (6), and Control (9).

Table 3.6. Literature summary regarding reported efficacies for Thymovar® including calculation method used.

Reference	Reported Efficacy	Efficacy Calculated with:
Bollhalder 1999	85-97%	Finishing treatment of sprayed oxalic acid
Marinelli et al. 2000	97.10%	Finishing treatment of Perizen®/Apitol®/queen caging
Rademacher and Radke 2001	72% singles 94% doubles	Not specified
Baggio et al. 2004	96.9 ± 0.73%	Finishing treatment of oxalic acid/Perizen/queen caging
Gerritsen and Cornelissen 2006	93%	Finishing treatment of coumaphos
Akyol and Yeninar 2008	96.91%	Varroa infestation in alcohol wash
Berg and Shurzinger 2008	97.6 ± 2.2%	Finishing treatment of trickled oxalic acid
Kutukoglu et al. 2012	78.4%-81.8%	Varroa infestation in alcohol wash

Figures

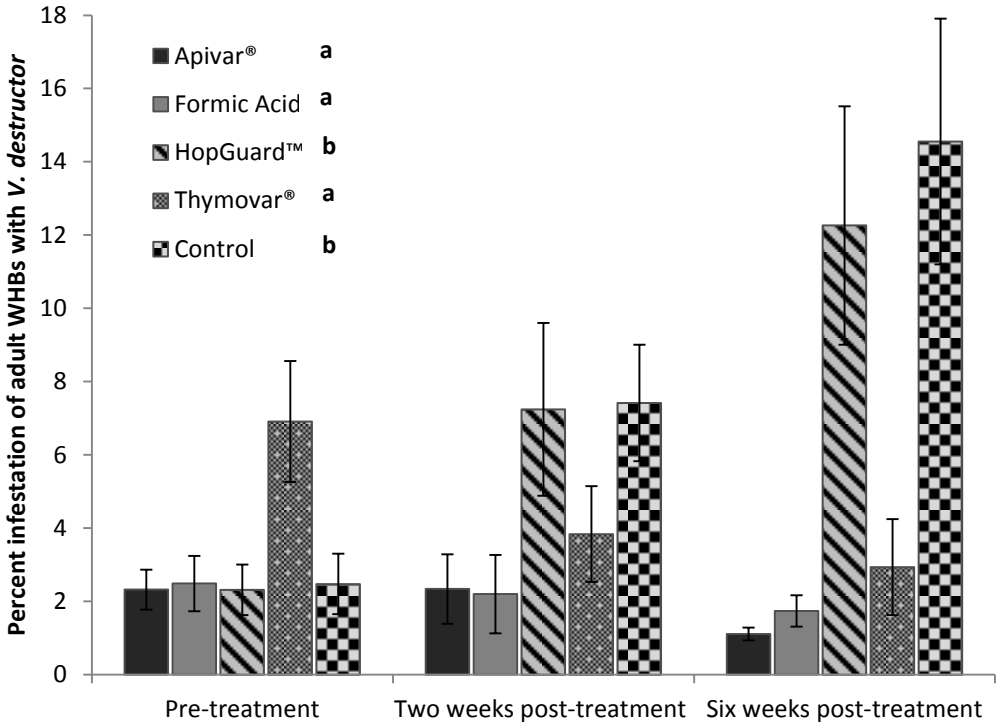

Figure 3.1. Average (± SE) percent infestation of adult WHBs with *V. destructor* in response to treatments throughout fall 2011 trial. Treatments followed by different letters vary significantly over time according to repeated measures ANOVA followed by Tukey means separation ($p<0.05$). The number of colonies pre-treatment was: Thymovar® (7) and other groups: (8). Due to queen loss, the number of colonies post-treatment was: Apivar® (7), formic acid (5), HopGuard™ (8), Thymovar® (6), and control (8).

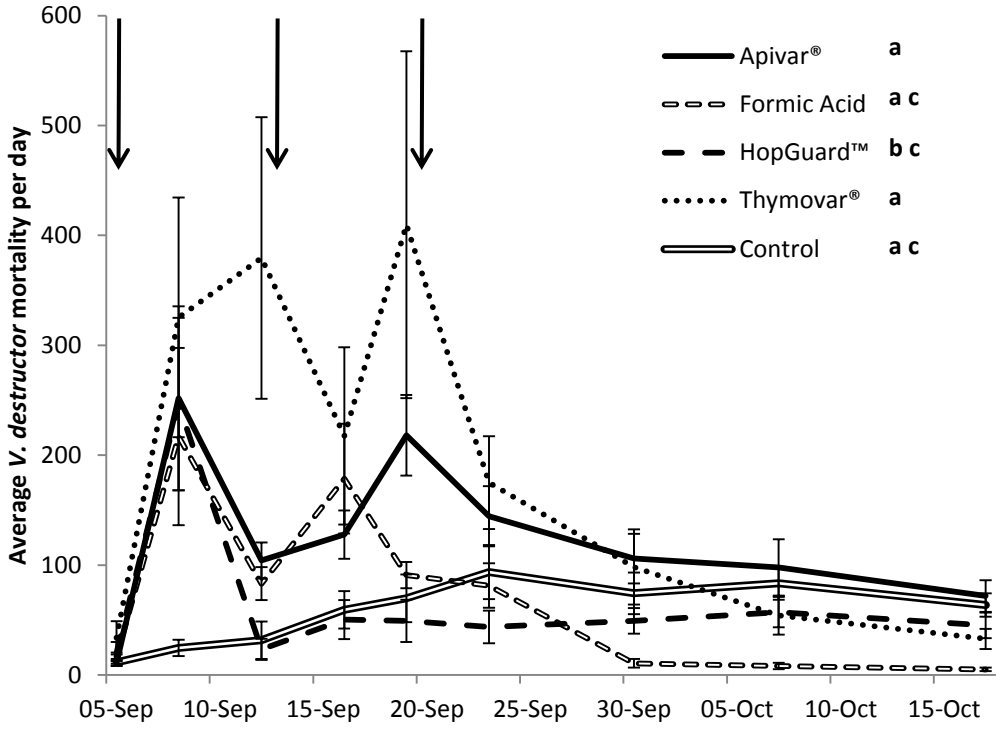

Figure 3.2. Average *V. destructor* mortality per day (± SE) in response to treatments throughout the fall 2011 trial. The arrows indicate from left to right: (1) application of all treatments (2, 3) reapplication of formic acid. Treatments followed by different letters vary significantly over time according to repeated measures ANOVA followed by Tukey means separation ($p<0.05$). The number of colonies was: Apivar® (7), formic acid (5), HopGuard™ (8), Thymovar® (6), and control (8).

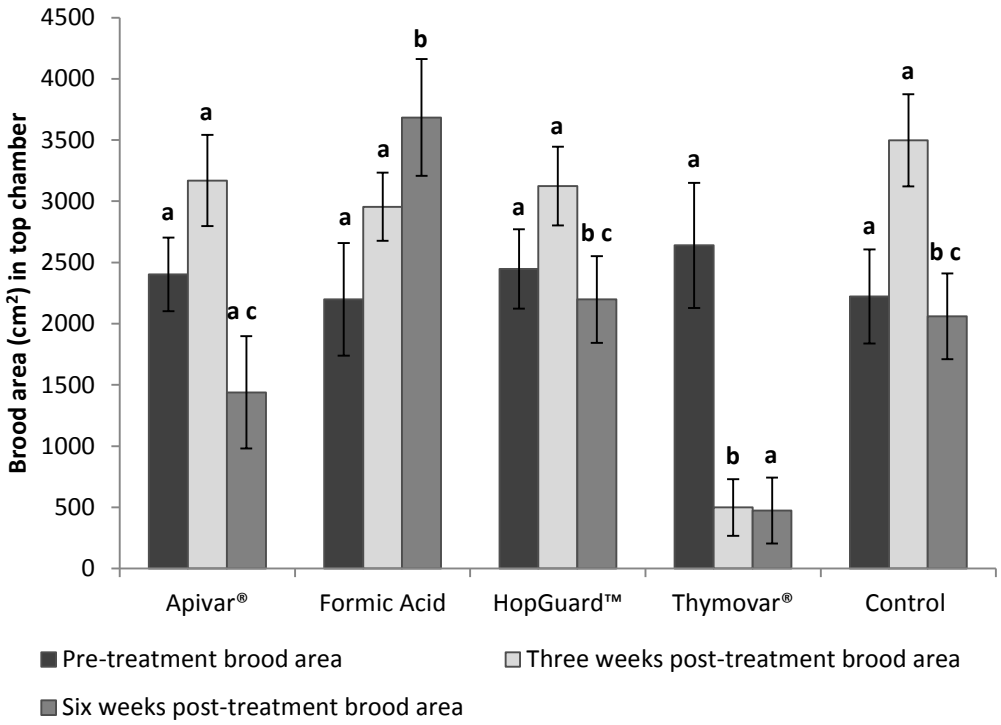

Figure 3.3. Average brood area (± SE) of the top brood chamber throughout the spring 2012 trial. Different letters indicate significant differences within each parameter at each sampling date (Tukey, $p<0.05$). The number of colonies pre-treatment was: control (9), and other groups (8). Due to queen loss, the number of colonies post-treatment was: Apivar® (8), HopGuard™ (7), Formic Acid (5), Thymovar® (6), and Control (9).

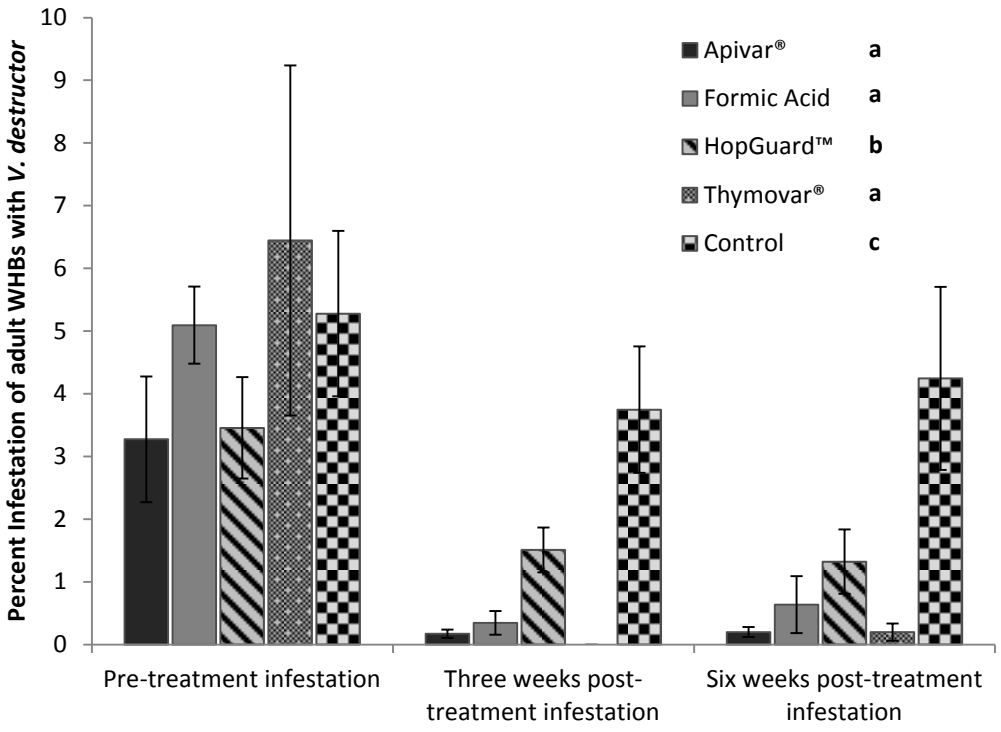

Figure 3.4. Average percent infestation (± SE) of adult WHBs in response to treatments throughout the spring 2012 trial. Treatments followed by different letters vary significantly over time according to repeated measures ANOVA followed by Tukey means separation ($P<0.05$). The number of colonies pre-treatment was: control (9), and other groups (8). Due to queen loss, the number of colonies post-treatment was: Apivar® (8), HopGuard™ (7), Formic Acid (5), Thymovar® (6), and Control (9).

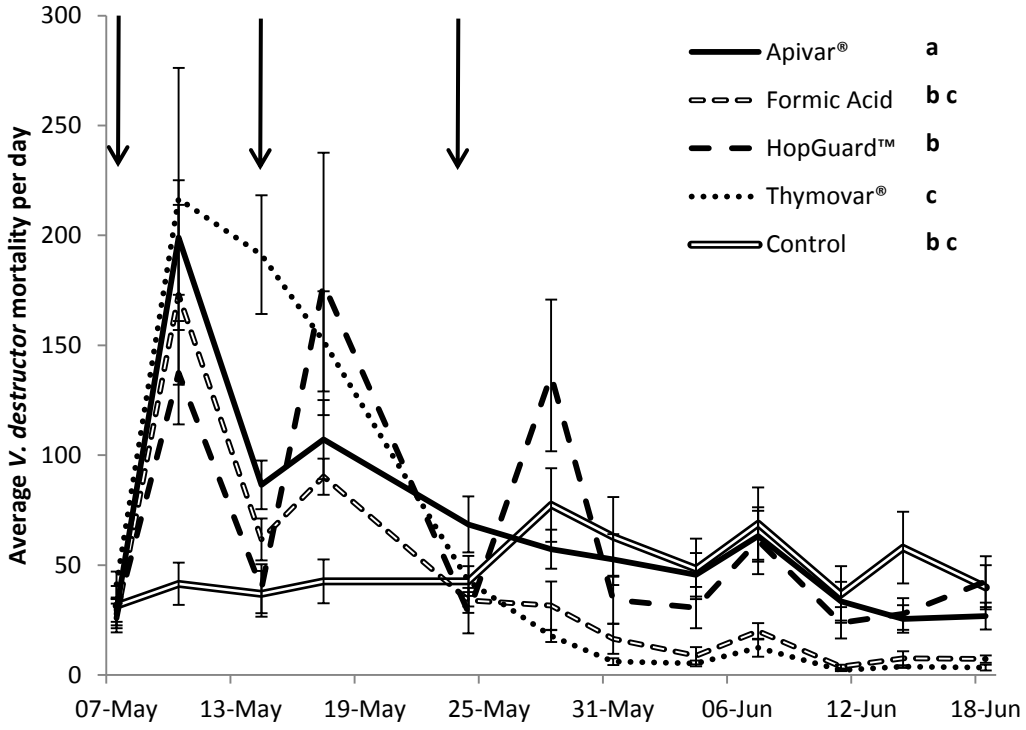

Figure 3.5. Average *V. destructor* mortality per day (± SE) in response to treatments throughout the spring 2012 trial. The arrows indicate from left to right: (1) application of all treatments (2) reapplication of HopGuard™ and formic acid (3) reapplication of HopGuard™, formic acid, and Thymovar®. Treatments followed by different letters vary significantly over time according to repeated measures ANOVA followed by Tukey means separation ($p<0.05$). The number of colonies was: Apivar® (8), HopGuard™ (7), Formic Acid (5), Thymovar® (6), and Control (9).

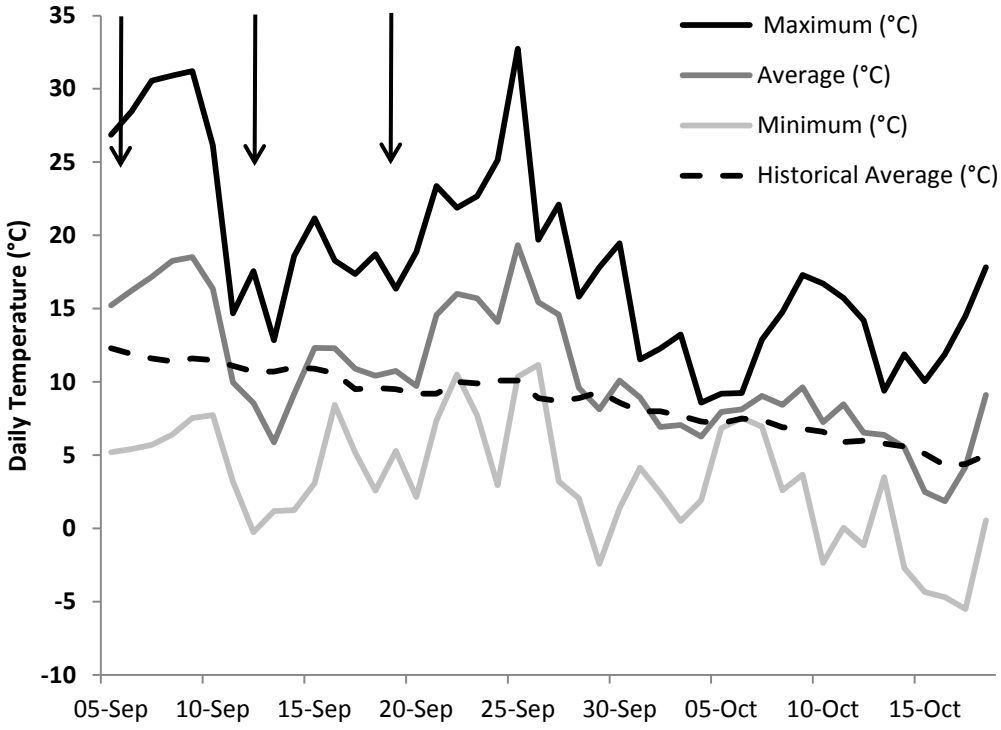

Figure 3.6. Maximum, average, minimum, and historical average daily temperature (°C) throughout the fall 2011 trial. The arrows indicate from left to right: (1) application of all treatments (2, 3) reapplication of formic acid.

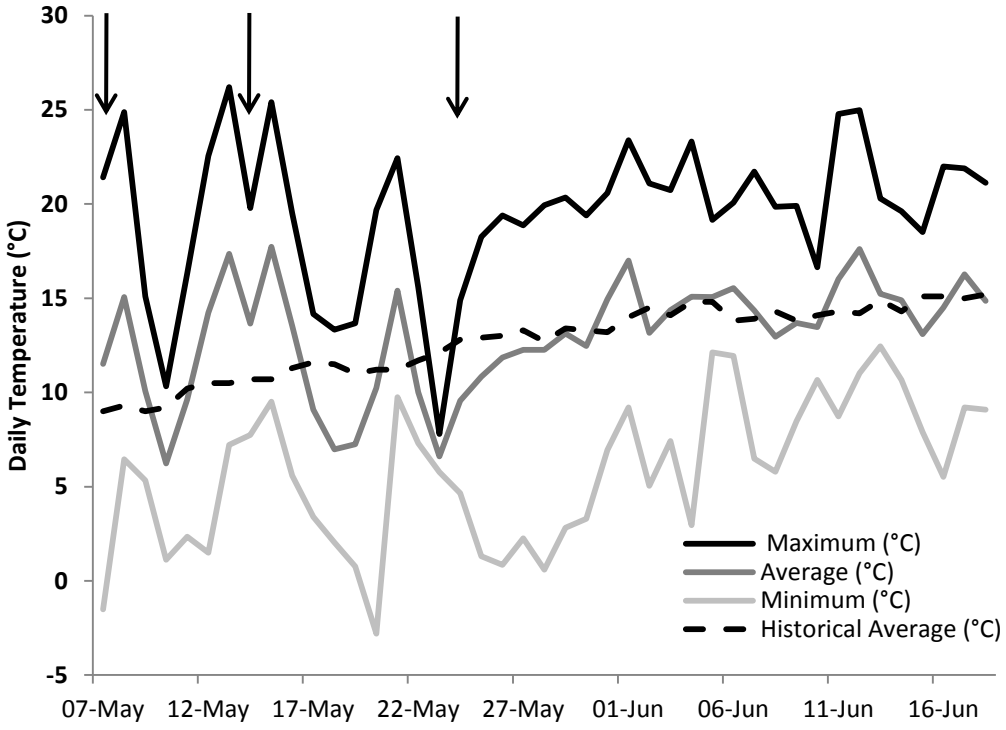

Figure 3.7. Maximum, average, minimum, and historical average daily temperature (°C) throughout the spring 2012 trial. The arrows indicate from left to right: (1) application of all treatments (2) reapplication of HopGuard™ and formic acid (3) reapplication of HopGuard™, formic acid, and Thymovar®.

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4. Chapter Four: General Discussion

4.1. Introduction

The Western Honey Bee (WHB), *Apis mellifera* L., is valued throughout the world as a managed pollinator and is an essential component of much global food production (Klein et al. 2007). Without rigorous management, *Varroa destructor* Anderson and Trueman 2000 (Acari: Varroidae) parasitism causes mortality of WHB colonies (Guzman-Novoa et al. 2010). *Varroa destructor* also vectors WHB viruses that are harmful to WHB colonies and may lead to over-wintering mortality (Dainat et al. 2012). Integrated Pest Management (IPM) is a strategy aimed at discontinuing prophylactic treatments of miticides through the judicious use of monitoring methods, economic thresholds, and management tactics so that miticide application for *V. destructor* is only performed when necessary (Delaplane et al. 2005). Tactics for the management of *V. destructor* include genetically tolerant WHBs, and use of miticides such as essential oils, organic acids, and synthetic miticides (Rosenkranz et al. 2010). Canadian populations of *V. destructor* have twice developed resistance to synthetic miticides, firstly to the pyrethroid Apistan® and secondly to the organophosphate Checkmite+™ (Currie et al. 2010). Currently, Apivar® is highly effective as a synthetic miticide for IPM of *V. destructor* in Canada (Nasr et al. 2010). However, in the event that Canadian populations of *V. destructor* develop resistance to Apivar®, new management tactics for *V. destructor* IPM systems are

needed. Alternative miticides containing essential oils or organic acids, as well as synthetic miticides with new chemistries would be valuable additions to IPM for *V. destructor.*

4.2. Laboratory assessment of new synthetic miticides for *Varroa destructor*

The objective of the laboratory bioassay used in chapter two was to assess the activity of synthetic miticides with new chemistries in relation to *V. destructor.* By screening synthetic miticides that are active against other acarids, the most promising compounds for further investigation were determined. The glass vial bioassay was an effective method of quantifying the LC_{50} for each synthetic miticide. Four of the five synthetic miticides bioassayed (Apollo®, Floramite®, Forbid®, and Shuttle®) had LC_{50}s for *V. destructor* under 10% of the miticide formulation and are candidates for additional analyses. The effect of Forbid® on *V. destructor* should certainly be examined further as studies have indicated its active ingredient spiromesifen may cause infertility of a related acarid (Irigaray and Zalom 2006). A similar effect on *V. destructor* would provide an important beneficial effect, enhancing the effectiveness of spiromesifen in IPM programs. Additionally, a recent patent application was filed for the use of spiromesifen to manage *V. destructor* (Fougeroux 2012).

To remove phoretic *V. destructor* from WHBs for use in the laboratory bioassay, a larger, modified version of the sugar roll monitoring method

described by Macedo et al. (2002) was very effective at harvesting hundreds of *V. destructor* at once. However, this method did create clouds of icing sugar in the laboratory, and Macedo et al. (2002) suggest that the icing sugar may reduce *V. destructor* longevity. A modified version of the ether roll can also be used to collect phoretic *V. destructor* for bioassays (Emsen et al. 2007); it would be interesting to determine which collection method results in increased *V. destructor* survival in laboratory bioassays.

The laboratory bioassay results were possibly affected by the heterogeneity of the probit models. This was likely due to the different genetics and age structure of the *V. destructor* populations used over the months that the bioassays were being conducted. One goal for future research is to increase the efficiency of the bioassay so that more bioassays could be conducted within a day. An additional option to reduce variability of the *V. destructor* population would be to maintain a population of adult WHBs infested with *V. destructor* in a package WHB cage for 3-4 days. Subsequently, all *V. destructor* harvested would be within a similar age cohort over 3-4 days old. Finally, *in vitro* propagation would undoubtedly increase accessibility and homogeneity of the *V. destructor* population; the lack of an *in vitro* method for *V. destructor* has previously been identified as a significant obstacle to successful miticide development (Dietemann et al. 2012).

For future bioassays, including another synthetic miticide known to cause significant *V. destructor* mortality within colonies is recommended. A commercial formulation of amitraz, the active ingredient of Apivar® strips, would be ideal. Thus, future work could assess effectiveness of additional synthetic miticides in relation to a known effective miticide using the systems set in place for this bioassay.

Further research regarding investigation of Apollo®, Floramite®, Forbid®, and Shuttle® for *V. destructor* management in WHB colonies is warranted. Requirements for synthetic miticides effective within *V. destructor* IPM include: 1) no significant side-effects on WHB colonies; 2) no residues in honey, and preferably no residues in wax; and 3) extended activity in WHB colonies causing significant *V. destructor* mortality over several weeks. One efficient and economical way to evaluate synthetic miticides within WHB colonies is to use a four-frame cardboard nucleus hive fitted with a screened bottom board. Population effects are more evident in a small colony; furthermore, it is more economical to dispose of a small cardboard colony in the event of wax and honey residues, and the number of replicates can be maximized.

Other studies have identified promising *V. destructor* miticides such as fungal pathogens or rotenone following laboratory assessment, but subsequent field trials showed that they were unsuitable in WHB colonies due to the rigorous requirements for use in WHB colonies (Meikle et al. 2012; Satta et al. 2008).

Therefore, we are cautiously optimistic that at least one of the four synthetic miticides found to cause *V. destructor* mortality will prove to be safe and efficacious within WHB colonies and contribute to future IPM of *V. destructor*. The study in chapter two is the first evaluation of these synthetic miticides against *V. destructor* and is a valuable addition to research initiatives for new *V. destructor* management options.

4.3 Field evaluation of alternative miticides for *Varroa destructor*

Chapter three is the first study concerning the application of two miticides, HopGuard™ and Thymovar®, to WHB colonies in Alberta. HopGuard™ is formulated in cardboard strips saturated with organic acids extracted from hops (*Humulus lupulus* L.). Thymovar® consists of cellulose wafers containing the essential oil thymol. A rigorous field-based experimental design allowed determination of side-effects on WHBs and efficacy of HopGuard™ and Thymovar® on *V. destructor* in comparison to industry standard treatments, and within the spring and fall treatment windows of Alberta. The registered industry standard treatments used were formic acid, an organic acid miticide, and Apivar®, a synthetic miticide containing the active ingredient amitraz. The trial was replicated in fall 2011 and spring 2012.

The number of replicates for each treatment in this study (although high compared to other studies of this nature) had to be kept reasonable (eight

colonies in each of the five treatment groups) so that the colonies could be assessed for vigor, which is labor intensive. Therefore, the sample size is too small to confidently draw conclusions regarding qualitative (yes/no) data such as queen survival or wintering ability. It would be interesting to conduct a larger study with approximately 20 colonies per treatment group to assess the effect of treatments on wintering ability.

4.3.1. Evaluation of HopGuard™

This is the first study regarding the use of HopGuard™ in Canada, and the first report worldwide involving the application of HopGuard™ to colonies with brood. In this study, no negative effects on WHBs were associated with HopGuard™ treatments in either trial. HopGuard™ was applied once in the fall 2011 trial and weekly for three weeks in the spring 2012 trial. In both trials, applications of HopGuard™ resulted in elevated *V. destructor* mortality in comparison to the control for three days, but applications were insufficient to provide any effective long-term management significantly different from the control. The WHBs were observed removing the cardboard strip within a few days, which is consistent with other findings (Skinner et al. 2001).

HopGuard™ is a promising addition to the organic acid group of miticides. This study demonstrates that it can be capable of causing initial *V. destructor* mortality within WHB colonies. However, this study also shows that HopGuard™

is limited by its delivery system. Cardboard strips are simply not feasible within a WHB colony (Skinner et al. 2001); the WHBs are disposed to remove them through chewing as part of regular colony maintenance. Hypothetically, additional applications of HopGuard™ (likely at least six) could increase efficacy to a reasonable level, but such a demanding treatment schedule is unlikely to be favored by beekeepers. Further research should focus on a delivery system for HopGuard™ that provides more lasting activity than cardboard strips. Examples of methods used to deliver miticides to WHBs while providing extended release include plywood inserts (Lubinevski et al. 1988), absorbent pads (Calderone 2010), gel matrices (Matilla and Otis 2000), plastic strips (Floris et al. 2001), vermiculite blocks (Calderone 1999), and cellulose wafers (Baggio et al. 2004).

4.3.2. Evaluation of Thymovar®

Thymovar® was not associated with any negative effects on WHBs in fall 2011, but drastic brood reduction was observed in response to Thymovar® treatments in spring 2012. As thymol-based products are temperature dependent, it can be difficult to achieve adequate evaporation within the colony without causing negative effects on WHBs (Imdorf et al. 1999). Negative effects on brood production in response to thymol-based treatments have been observed before (Floris et al. 2004), but not to the extent seen in this study. As spring brood production is closely correlated with summer colony strength and honey production (Szabo and Lefkovitch 1989), spring brood reduction of this

magnitude is not acceptable to beekeepers. However, this study shows that Thymovar® can be safely used in WHB colonies during the fall treatment window, and is efficacious within that window.

Due to the unseasonably warm conditions experienced in fall 2011, and the temperature dependency of thymol-based products, further research should determine what the effect of Thymovar® would be in an average or unseasonably cold fall. Additionally, the use of single brood chambered colonies is continually rising within Alberta; further research could also assess the performance of Thymovar® in single colonies.

4.4. Implications for Integrated Pest Management

IPM is a useful strategy that has not been well defined within the beekeeping industry. IPM as developed by Luckmann and Metcalf (1982) was for insect pests of field crops and included the following steps: (1) pest identification, (2) knowledge of biology, (3) monitoring of population levels, (4) determination of treatment thresholds, (5) tactic selection, and (6) evaluation of results. The adoption of IPM strategy in *V. destructor* management is a testament to the applicability of this theory, but it does require some industry specific modification. Given the nature of WHB biology, honey production seasonality, the specificity of treatment windows, economic considerations, and seasonal variability in treatment efficacy, I propose that a step regarding

treatment timing be added to the IPM strategy for WHB and *V. destructor*. Furthermore, it would provide clarity if the monitoring step included the steps of pest identification and evaluation of results. Pest identification suggests visual detection of the pest, but unfortunately it has been my experience that once *V. destructor* populations can be detected visually in the colonies without the aid of a monitoring technique, they have already surpassed the economic threshold. Evaluation of results needs to be incorporated as part of monitoring because beekeepers rarely monitor their colonies after treatments have been carried out, and consequently are rarely aware of the effectiveness of treatments within their operation. Additionally, if it was emphasized that monitoring is also necessary after treatment, there would be more time to appropriately address resistance issues when they do arise. Therefore IPM for *V. destructor* would include the following steps: 1) treatment timing, 2) monitoring for the presence of *V. destructor*, as well as the level of *V. destructor* prior to and after treatment, 3) consultation with regionally and seasonally specific economic thresholds, and 4) tactic selection.

4.4.1. Treatment timing

The results of chapter three and a review of recent literature has led me to conclude that treatment timing should be included as an integral component of IPM for *V. destructor*. The two treatment windows in Alberta are separated by economically important periods: honey production and wintering. Therefore,

beekeepers need to evaluate their risk before selecting an appropriate time to apply treatments for *V. destructor*. A shift in treatment timing is currently evident whereby beekeepers are predominately applying Apivar® in spring (Nasr 2012). The reasons for the shift in treatment timing are summarized in Table 4.1. Recent research (vanEngelsdorp et al. 2008, Locke et al. 2012) suggests that fall treatments may be associated with risk as viral loads do not immediately decline with reduced *V. destructor* population through miticide treatments. Therefore, it may be prudent to consider a spring synthetic miticide treatment as an economical and effective way to manage *V. destructor* populations while ensuring that viral loads are low prior to winter. A shift of synthetic miticide treatments to spring is likely correlated with increased use of non-synthetic treatments during the fall. As chapter three shows that Thymovar® treatments should be limited to the fall window, it is likely that Thymovar® will function well in tandem with spring synthetic miticide treatments.

4.4.2. Monitoring methods

As previously stated, IPM is more effective if beekeepers monitor for the presence of *V. destructor,* and monitor the *V. destructor* population before and after treatments. Therefore, successful IPM of *V. destructor* is reliant upon methods that encourage beekeepers to monitor frequently as seasonal *V. destructor* populations develop. For instance, instead of monitoring *V. destructor* with sticky traps or the alcohol wash method, the *Varroa* Hand Shaker (Nasr and

Williamson 2010) can be used by beekeepers because it is efficient and field-based.

As discussed in chapter three, seasonal *V. destructor* population cycling between the phoretic phase and reproductive phase (Martin 1998) needs to be taken into account in addition to regional considerations when interpreting infestation data obtained with methods such as the *Varroa* Hand Shaker. Therefore, an important goal of future extension efforts should be to educate beekeepers regarding seasonal *V. destructor* dynamics in relation to WHB population dynamics in the region. Future research should ensure that economic thresholds for infestation methods take seasonal *V. destructor* population cycling into consideration. Additionally, while monitoring methods are well developed, practical systems for monitoring with details such as the number of colonies per apiary to be monitored should be a goal of future studies.

4.4.3. Thresholds

The use of regionally specific economic thresholds allows beekeepers to manage *V. destructor* by applying treatments when they are needed to reduce the *V. destructor* population to avoid damage to WHB colonies, rather than treating on a predefined schedule without monitoring. While the objective of chapter three was not to specifically evaluate economic thresholds, chapter three shows that the average pre-treatment *V. destructor* infestation and *V. destructor* mortality for the fall 2011 trial was above the August threshold set by

Nasr et al. (2008), but below the thresholds given by Delaplane and Hood (1999) and Strange and Sheppard (2001). Considering that the *V. destructor* infestation and *V. destructor* mortality in the untreated control colonies climbed to well above the economic thresholds set by Delaplane and Hood (1999) and Strange and Sheppard (2001) within two weeks of treatment, the conservative threshold set by Nasr et al. (2008) is appropriate for Alberta.

As previously discussed, viral dynamics are relevant when implementing treatment thresholds and future research should re-examine currently used thresholds with consideration given to the effect of viruses on WHB colony damage. Additionally, Calderone (2010) suggests a shift from currently used economic treatment thresholds to economic threshold ranges that define the *V. destructor* population that can be effectively managed given the efficacy of a specific product. A product-specific economic threshold range would be beneficial when using non-synthetic miticides which may have limited or variable efficacy, thus ensuring that the appropriate product is used within each window of application and the risk of colony decline prior to winter or honey production is minimized. Calderone (2010) further suggests taking temperature and colony-to-colony variability of treatments into consideration when defining the economic threshold range. Future research could focus on recommending a threshold range for various non-synthetic miticides; it is possible that such a threshold range would also increase the use of non-synthetic miticides such as essential oils or organic acids.

4.4.4. Management tactics

4.4.4.1. Genetically tolerant strains of WHBs

While WHBs that are genetically tolerant to *V. destructor* are a viable non-chemical component of *V. destructor* IPM (Delaplane et al. 2005), and have functioned well within modern beekeeping regimes (Danka et al. 2012a), the use of genetically tolerant WHB stocks among beekeepers varies. To increase beekeeper acceptance of tolerant WHB stocks, beekeepers need to be assured that tolerant stocks do not have compromised traits such as honey production or gentleness (Rinderer et al. 2010). The necessity of frequent requeening requires a sustainable source of queens (Danka et al. 2012a), but the climate of Alberta restricts early queen production; consequently, commercial beekeepers are unable to raise sizeable quantities of their own queens and are reliant upon the importation of queens from international suppliers. Alberta imports approximately 125,000 queens a year from Hawaii (M. Nasr Personal Communication), but research suggests that because Hawaii was free from *V. destructor* until 2008, commercial WHB Hawaiian stocks do not express genetic tolerance to *V. destructor* (Danka et al. 2012b). Therefore, suppliers of mass quantities of genetically tolerant queens are needed so that genetically tolerant WHBs can be a valuable component for IPM of *V. destructor* within Alberta.

4.4.4.2. Essential oils and organic acids

Non-synthetic miticides such as essential oils and organic acids remain

integral to *V. destructor* IPM as they can lengthen the time between synthetic miticide treatments and manage resistance to synthetic miticides (Delaplane et al. 2005). This study shows the potential of HopGuard™ within WHB colonies pending a change in delivery system, and that Thymovar® is a viable treatment option for the fall treatment window. Substantial progress has been made regarding efficient delivery systems for essential oils and organic acids (Rosenkranz et al. 2010) and further research could refine currently used delivery systems and explore new delivery system options. As previously mentioned, future work should focus on the development of product-specific threshold ranges for essential oil and organic acid-based miticides to facilitate greater use of these miticide products.

4.4.4.3. Synthetic miticides

A review of *V. destructor* literature shows that many researchers are critical of the further development of synthetic miticides given the associated resistance issues and wax residues (Dietemann et al. 2012). However, they remain the only consistent way of managing *V. destructor* (Delaplane et al. 2005). An alternative way to view the development of synthetic miticides is to consider amitraz (used in Canada as Apivar®), which is from a novel class of compounds called formamidines, because it has been associated with few of the other problems that have been related to the use of pyrethroids and organophosphates. No residues of amitraz are apparent in wax or honey (Martel

et al. 2007), effects on queens and drones have not been reported, and chapter one summarized that less resistance has been reported worldwide to amitraz than to the pyrethroids and organophosphates. Moreover, while US resistance was first reported to amitraz in 2000 (Elzen et al. 2000), it is still used effectively in the USA (Danka et al. 2012a). In summary, acceptance of synthetic miticides as a viable component of *V. destructor* IPM would be more likely if amitraz were viewed as exemplar of synthetic miticides used in WHB colonies rather than the controversy-plagued organophosphates and pyrethroids. Amitraz is effective against *V. destructor,* safe for WHBs, and leaves no residues in honey or wax; if the new synthetic miticides identified in Chapter two function in a similar manner, they could be integral components in successful *V. destructor* IPM.

4.5. Conclusions

IPM of *V. destructor* continues to be an essential area of focus in WHB research. The importance of treatment timing is emerging as an integral component of IPM. Monitoring methods are being streamlined and calibrated so that they can be efficiently used by beekeepers in conjunction with economic thresholds. Economic thresholds are evolving according to region, monitoring method, and viral and WHB population dynamics; their effectiveness may also increase with product specificity. Wide arrays of management tactics are available to beekeepers including genetically tolerant strains of WHBs and management tools such as essential oils, organic acids, and synthetic miticides.

This research adds to current IPM of *V. destructor* on the Canadian prairies as it identifies new organic acids and essential oils for use in WHB colonies. This study also demonstrates the potential for new synthetic miticides to contribute to *V. destructor* IPM and facilitates future synthetic miticide development. Ongoing integration of IPM and beekeeping management practices will promote the maintenance of healthy colonies of WHB, the production of safe, quality honey, and ensure sustainable management of *V. destructor*.

Tables

Table 4.1. Evaluation of window of application of synthetic miticide treatments regarding cost and risks in Alberta

Parameter	Spring Treatment	Fall Treatment
Cost	Less because only colonies that survive the winter are treated	More because colonies that will die over the winter are treated
Brood chamber that miticide strips must be placed in	Top chamber as the WHBs have moved up into it (less labor intensive)	Bottom chamber as the WHBs have shifted down through the colony (more labor intensive)
Risk of *V. destructor* population recovery by the next treatment window	High as there are several intervening brood cycles between spring and fall	Low because there are few brood cycles over the winter, and *V. destructor* die in the extended phoretic phase over the winter
Risk to honey production	Low because summer colonies should have low levels of *V. destructor*	High because *V. destructor* populations may recover by the following summer
Risk to overwintering because of the *V. destructor* population	High because *V. destructor* populations may recover by fall	Moderate because *V. destructor* populations should be low, but winter WHBs may have been damaged
Risk to overwintering because of viral dynamics	Low because viral loads likely have not had time to build up	High because viral loads have not decreased yet

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