Increased Sustainability Managing Bee Pests (Varroa Mites and Nosema) for Alberta Beekeepers

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Appendix 3.

Effectiveness of Miticides against the Ectoparasitic Mite, *Varroa destr*uctor of the Honey Bee, *Apis mellifera*

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Abstract:

Varroa destructor is an ectoparasitic mite that leads to serious losses in honey bee colonies. Low efficacy and development of mite resistance to miticides has increased the demand for innovative, effective treatments that will exhibit high efficacy of controlling Varroa mites, while minimizing side effects on the host's fitness, *A. mellifera*. In this investigation, glass vial and micro-application bioassay methods were developed to evaluate acute contact toxicity (LD_{50} and LC_{50}) of commercial formulated products (FPs) (n= 11) and their active ingredients (AIs) (n= 11) on Varroa mites and honey bees. Adult mites or bees either topically treated with different concentrations of FPs or AIs or exposed to compound concentrations in glass vials. In the 2016 and 2017 experiments, mite mortality was evaluated after 6 and 18 hours (h) and 4 and 24 h, respectively, and bee mortality was assessed after 24h. Amitraz was used as a positive control. In field trails, Varroa-infested honey bee colonies were established with new mated queens; and bee populations, brood and mite level were equalized. All colonies were randomly assigned into treatments to further evaluate the effectiveness of the laboratory tested FPs or AIs. Collectively, our findings suggest that some four active ingredients from Tetronic acids, Quinazolines and Benzoylacetonitriles groups have potential to manage Varroa mites in honey bee colonies. Further development of ab application method for these compounds will conducted to develop a commercial product to be used by beekeepers.

Key Words: *Apis mellifera*, *Varroa destructor*, Miticides, Toxicity, LD_{50}, LC_{50}, Bioassay.

Introduction:

Globally there are many species of bees and other insects that play an important role in plant pollination. This pollination service is vital to the maintenance of both wild plant communities (Loreau et al. 2001; Naeem et at. 1994) and agricultural crop productivity (Costanza et al. 1997; Kearns et al. 1998). It is estimated that the value of insect pollination to global agriculture is approximately $197 billion per year. Despite the wide diversity of plant pollinators, the honey bee, *Apis mellifera* is the world's most important managed pollinator of agricultural crops. Approximately one third of the food that we consume each day relies on pollination mainly by bees. Indeed, Klein et al. (2007) stated that honey bees' contribution to world food production is indispensable.

According to U.N. – F.A.O. data, the world's number of bee colonies reached over 90 million in 2016. Canada has 767,000 of those colonies and the annual value of honey bees' contributions to pollinated crops is estimated to be $2.5 billion (AAFC, 2017). Forty percent of Canada's colonies are located in Alberta (Canada Stats 2017). Alberta's the annual contributions of bees to pollinated crops including hybrid canola seed is about $1.2 billion. Although pollination is one service, honey bees produce many highly valued products such as honey, wax and pollen. Most of these products are marketed by beekeepers for human consumption. In 2016 Canada produced approximately 41.8 metric tons of honey valued at approximately $157 million (Statistics Canada 2017). In 2016 Alberta beekeepers produced over 17.8 metric tons of honey valued at approximately $85 million according to Statistic Canada (2017).

In recent years managed honey bees have come under serious health threats, which has resulted in beekeepers losing a significant number of bee colonies. In Canada, beekeepers reported substantial colony losses in the winters of 2006-2009. The reported annual winter losses have averaged 30%, which is double the long term average winter losses of 15% (CAPA 2007, 2008 and 2009). In Canada research initiatives investigating bee colony losses suggested that various regions of the country have attributed different stressors, or a combination of interacting stressors that may have caused high winter losses (Currie et al.

2010; Guzmán-Novoa, et al. 2010). Similar winter losses were also reported in the USA (vanEngelsdorp et al. 2009). However, these losses were attributed to Colony Collapse Disorder (CCD). Several other contributing causes for CCD have been suggested including bee parasitation by Varroa mites (LeConte et al. 2010; vanEngelsdorp et al. 2012), Nosema (Higes et al. 2008), viruses (Cox-Foster et al. 2007; Carreck et al. 2010), poor nutrition (Di Pasquale et al. 2013), and pesticides (Mullin et al. 2010; Wu et al. 2011).

In Alberta, beekeepers suffered high losses of honey bee colonies during the winters of 2007-2009. They reported honey bee winter losses from 30-45% per year. As a result, Alberta Agriculture and Forestry established a surveillance program to determine possible causes of high bee losses. Alberta Agriculture and Forestry found that Varroa resistance to applied Checkmite+™ was the main cause of high losses. Additionally, a combination of one or more factors was implicated in high winter losses. These factors include lack of winter bees in wintering bee colonies, very cold winter temperatures, unpredictable cold spring temperatures, and high levels of Nosema infections (Hartman and Nasr 2007 and 2008).

In response to these high losses of bee colonies, Alberta Agriculture and Forestry supported the registration of a new miticide, Apivar® to control Varroa mites. It has also been recommended that beekeepers improve their monitoring of the Varroa population throughout the year, treat on time and improve their management practices to ensure healthy colonies going into winter. Mitigating high annual colony losses through increased splitting of surviving stock or purchasing replacement colonies have been also encouraged. Consequently beekeepers have become successful in reducing winter losses to 15-20% per year since 2011 and have been able to replace all dead colonies and annually increase their managed bee colonies by 2-5%.

Despite this recent success, Alberta beekeepers have identified the Varroa mite, *Varroa destructor* as their single most serious problem causing colony losses today. It is considered the most prevalent pest in North America. This mite is an ectoparasite that threatens bee colony health by feeding on the

hemolymph of immature and mature bees, and transmitting viruses that contribute to colony loss. In order to control Varroa mites, beekeepers use several miticides to keep their populations below the treatment thresholds in managed bee colonies. In Canada, beekeepers use registered compounds to control Varroa mites such as Apivar®® (Amitraz, Formamide), Apistan® (tau-fluvalinate, Pyrethroids), CheckMite+™ (Coumaphos, organophosphate), Apiguard® (Thymol, Monoterpenoid), and Formic and Oxalic acids (organic acids).

Unfortunately, widespread reliance on miticides such as Pyrethroids, Formamides and Organophosphates, as well as extensive dependence on miticide with the same mode of action such as Apistan® and Bayvarol® have increased resistance rates in mite populations in North America (Elzen et al. 1999), South America (Maggi et al. 2009) and Europe (Milani 1995). In addition to this, the long term use of synthetic miticides has led to the accumulation of residues in bee products (Martel et al. 2007). In an attempt to manage resistance to synthetic miticides, organic acids such as formic acid, oxalic acid and essential oil based miticides such as Thymovar, have been registered for use against Varroa mites. However, when beekeepers apply these organic acids and essential oil based miticides, they achieve variable results of mite control. To have acceptable results, these miticides have to be applied within a certain ambient temperature range and have no or a minimal brood present in bee colonies. Therefore, beekeepers have become reluctant to use these miticides to control mites.

In order to develop sustainable mitigation strategies for mite control, it has been suggested to increase the number of chemicals with different modes of actions to improve our options for treating colonies in the field. Currently, the Pest Management Regulatory Agency (PMRA) registered 31 groups of insecticides/acaricides based on target site and mode of action. However, to date, beekeepers have used 3 synthetic miticides that belong to 3 different chemical groups. Accordingly, there are more potential registered miticides to be assessed for activities against Varroa mites and bee safety.

To measure the activities of miticides, a glass vial bioassay that was initially developed by Plapp et al. (1987) to evaluate the resistance of the tobacco budworm and subsequently used for determining the resistance or toxicity of chemicals against *Halotydeus destructor* (Tucker) (Hoffmann et al. 1997), *Bemisia argentifolii* Bellows and Perring (Prabhaker et al. 1996), *Tetranychus urticae* Koch (Latheef and Hoffmann 2014), Varroa destructor (Elzen et al. 1999; Kanga et al. 2010; Vandervalk, 2013: Kamler et al. 2016) was used on both Varroa mites and honey bees. This assay determines the concentration of a tested chemical required to kill 50% of the population at a specific time (i.e. LC_{50}). The second bioassay that is used to determine the lethal dose (LD_{50}) is based on using a precision hand micro-applicator for Varroa mite and the honey bee. Successful miticides from the LC_{50} and LD_{50} experiments that were effective at controlling Varroa mites with minimal harm to honey bees were selected for further testing in the field using bee colonies.

The main objectives of this research project were to assess the activities (LC_{50} and LD_{50}) of active ingredients (AI) and their PMRA registered Commercial Formulated Products (FP) against Varroa mites and honey bees under laboratory conditions. The AI and FP that have exhibited activities against Varroa mites and are safe for bees were then subjected for further testing under field conditions using honey bee colonies. The field test will determine dose response of applied potential miticides on mortality of Varroa mites and honey bees.

Materials and Methods:

The laboratory and field trials were carried out at the Crop Diversification Center North, Edmonton, Alberta, Canada (53.54 °N, 113.49 °W) in summer-autumn 2016 and 2017. All bees used in this bioassay study were provided from European honey bee (*A. mellifera*) colonies. The experimental bee colonies were managed using standard management practices. These colonies were not treated for mite control but they were treated for nosema using proper doses of Fumagillin according to the label.

Testing potential miticides:

The activities of 12 Formulated Products (FP) registered for use on plant mites by the Pest Management Regulatory Agency and their chemical groups that were assessed in 2016 and 2017 are presented in Table 1. The total FP and their chemical groups available for use are listed in Table 2. The active ingredient Amitraz and associated commercial formulated product, Mitaban, was used as a positive control in this experiment. All FPs, and their AIs were obtained from Sigma-Aldrich and other miticides' supply companies (Tables 1 and 2A, 2B). All tests were performed in a restricted laboratory under the fume hood. Operators had implemented all personal safety practices to protect themselves from any exposure to tested chemicals including wearing a fitted full-face respirator mask (6900, 3M^{TM}, USA) equipped with filters (60923, 3M^{TM}, USA), doubles nitrile gloves (Kimberly-Clark, USA) and lab coat.

A. Laboratory assay to determine activities of tested miticides against Varroa mites and bees.

<u>Experiments conducted in 2016</u>

Testing Varroa mites for resistance to Apivar® (Amitraz) and Apistan® (fluvalinate).

Before the experiments began, the evaluation of mite resistance to Apivar®® (Amitraz, Veto-Pharma, France) and Apistan® (fluvalinate, Wellmark International, ON, Canada) took place in summer 2016 and 2017 on mite infested colonies using the Pettis method (Pettis et al. 1998). This test was done to ensure that the collected mites did not have resistance to Amitraz that was used in the bioassay as positive (reference) control. In the Pettis method, a group of 150 worker bees were exposed to a piece of Apistan or Apivar® strip (1 X 2.5 cm) that was stapled on cardboard and placed in a 500 ml Mason jar (Bernardin, Canada) (Fig. 1). Jars filled with infected bees were incubated at 25ºC for 24 h to allow exposure of mites to Apivar. After 24 h, each jar was placed upside down on a piece of white paper and shaken up and down for 2 minutes. Dead mites fall through the screen on the white paper. The number of dead mites were counted. Ethanol (70%) was added to fill the jars half way. The jars were covered with their regular caps

and were agitated on a 175 rpm orbital shaker for 15 minutes. The jars with bees were poured into a 12-mesh strainer. Individual mites fell through the strainer and were subsequently collected in an 11 Litre Rubbermaid® basin. Bees were rinsed for 90 seconds with running water to ensure all mites were collected. Mites in the basin were counted. The efficacy was calculated by the number of mites that initially fell on the white paper before the bees were killed, divided by the total number of mites killed (Note that the total mites killed = white paper mite count + mite count from bee washing) and multiplied by 100.

Vial assay to determine LC_{50} of tested AIs and FPs on mites in 2016

Mite collection:

Mites for bioassay tests were collected from the colonies highly infested with mites that were not resistant to Apivar (Amitraz). Mites were collected using the carbon dioxide method as described by Bahreini and Currie (2015). In this method, 300-500g of highly-infested bees were placed in a Plexiglass box (8.5 X 14X 28.5 cm) fitted with a 12-mesh screen on the bottom. The box was then placed in a Rubbermaid container (12 X 20 X 35 cm). The container was agitated at 300 rpm for 10 min on a shaker while being exposed to CO_2 (5 L/min). Mites that fell onto the bottom of the Rubbermaid container were collected with the tip of a fine-tipped paint brush and placed in Petri dishes lined with a moist paper towel.

Vial assay procedure for mites:

Eight to ten Varroa mites were exposed to serial dilutions (0.0%, 0.0001%, 0.001%, 0.01%, 0.1 and 1%) of AIs or serial dilutions (0.0%, 0.01%, 0.1, 1% and 10%) of FPs. The 10 ml stock dilutions for each FP (10%) and AI (1%) were made in 15 ml Fisherbrand® polypropylene centrifuge tubes (Fisher Scientific, Canada) and agitated on a vortex mixer (VWR, USA) for a period of 2-3 min. Acetonitrile (Sigma-Aldrich, USA), acetone (Sigma-Aldrich, USA), chloroform (VWR, USA), xylene (Acrose, USA) and water were pre-tested to find a suitable solvent for candidate compounds. Acetone and water were chosen as solvents for the active ingredients and formulated products, respectively. Mitaban and Amitraz

served as positive control treatments for FPs and AIs, respectively, and untreated groups were considered as negative controls. Five replicates were used for each tested concentration.

The assigned 20 ml borosilicate glass scintillation glass vials (Cole-Palmer, QC, Canada) were treated with 0.5 ml of each concentration using a pipette. The glass vials were then rotated on a cold hot dog roller (Addcraft®, Hicksville, NY, USA) under a fume hood at room temperature for 2-3 hours until solvents completely evaporated and compounds homogenously coated the inner surface of vials (Fig. 2). Live mites were then introduced into the treated vials using a labeled new fine brush for each concentration to prevent cross-contamination.

After preparation and introducing Varroa mites, vials were placed in the incubator (She Lab, Sheldon Inc., OR, USA) at 25 ºC and 60% Relative Humidity (RH) for 6 h in the dark. Mite mortality was counted 6 h post-treatment and the surviving mites were then transferred into clean vials containing pink-eyed pupae for feeding (Fig. 3). Vials were then incubated at 25 ºC and 60% RH for additional 18 h in the dark. The temperature and relative humidity inside the incubators was monitored every 15 minutes using HOBO C-12 (Onset Computer Corporation, Bourne, MA) data loggers. Vials were removed out of the incubator and mite mortality was assessed to determine 24 h post-treatment kill. Mortality was assessed by gently probing mites using a fine-tipped paint brush where the motionless mites were considered dead.

Vial assay to determine LC_{50} of tested AIs and FPs on honey bees in 2016

Collection of bees:

In order to provide the newly-emerged bees for the honey bee vial bioassay, frames with emerging capped brood without any attendant workers were removed from healthy bee colonies with zero to <1% Varroa mite infestation. Each frame of capped brood was placed in a screened wooden emerging bee cage (7WX 26H X50L cm). Cages were placed in an incubator at 33 ºC overnight. Newly emerged bees were shaken

off the frame into a Rubbermaid container (12 X 20 X 35 cm). Bees were then exposed to CO_2 for 5-10 min until anesthetize.

Vial assay procedure for bees:

The toxicity of compounds to the honey bee was assessed using the Mason jar (500 ml) method (Riusech, 2017). A piece of plastic strip (2.54 x 11.43 cm) covered with 0.25 ml of the prepared concentrations was applied to one side of the strip. The treated plastic strips were placed in the chemical fume hood to dry. Then, 0.25 ml of the same concentration of the same tested product was placed on the other side of the strip and returned to the fume hood to dry.

Each treated dried strip was placed in the center of a 500 ml Mason jar. The strip was held in place by gluing one sugar cube to each side of the strip and onto the bottom of the jar using hot glue gun. Sugar cubes were also used for feeding the bees during the exposure time. A group of 107-109 (average 108±0.98) newly emerged bees were placed in each prepared Mason jar. All prepared Mason jars with bees were placed in an incubator at 25 ºC and 60% RH for 24 h in the dark. Dead bees were counted in each jar to determine bee mortality at tested concentrations after 24 h exposure.

Experiments in 2017

Determination of LC_{50} of tested AIs and FPs on mites in 2017

Vial Assay procedure:

All mites were sourced from colonies infested with mites that had no resistance to Apivar (Amitraz). Mites were prepared and collected following the same procedure used in 2016.

Vial assay procedure for mites:

The list of tested FPs and AIs is presented in Tables 2A, 2B. The 5 ml stock dilutions for each tested FP or AI were made in 15 ml Fisherbrand® polypropylene centrifuge tubes (Fisher Scientific, Canada) and agitated on a vortex mixer (VWR, USA) for a period of 2-3 min. Acetone and water were used as solvents for the AIs and FPs, except Acetonitrile for Clofentezine. Mitaban and Amitraz served as positive control treatments for FPs and AIs, respectively. Vilas with no treatments were used as a negative control.

Tested serial dilutions were 0.0%, 0.00001, 0.0001%, 0.001%, 0.01%, 0.1 and 1% of AIs and 0.0%, 0.000001, 0.00001, 0.0001%, 0.001%, 0.01%, 0.1, 1% and 10% of FPs. 20 ml borosilicate glass vials were treated with tested dilutions as described above in 2016 experiments. Overall nine AIs and nine FPs were tested (Tables 2A and 2B). Control treatments included applying 0.5 ml/vial of water, ethanol, Acetone, or Acetonitrile and a negative controls that includes solvents for each perspective tested product. Four replicates were used for each tested concentration including controls.

After treated vial preparation and seeding with 8-11 Varroa mites, vials were incubated (NorLake™, USA) at 33±1 ºC and 70±2% RH for 4 h in the dark. In 2017 the exposure time of mites to treated vials was only 4 h. Mite mortality was counted 4 h post-treatment and the surviving mites were then transferred into the 1.7 ml polypropylene centrifuge tubes (VWR, Canada) containing pink-eyed pupae for feeding. Tubes were then incubated at 33±1 ºC and 70±2% RH for 20 h in the dark. Mite mortality was assessed to determine 24 h post-treatment kill. Mortality was assessed by gently probing mites using a fine-tipped paint brush and the motionless mites were considered dead.

Vial assay to determine LC_{50} of tested AIs and FPs on honey bees in 2017

Jar assay procedure for bees:

The contact toxicity (LC_{50}) of compounds to the honey bee was evaluated using modified the Mason jar method. Sixty ml-Mason glass jars (Uline, Canada) were used to replace the 500 ml Mason jars used in 2016 to reduce the amount of applied tested products. The 60 ml Mason jars were treated with 1 ml of each concentration (0.0%, 0.00001, 0.0001%, 0.001%, 0.01%, 0.1 and 1%) of AIs or (0.0%, 0.000001, 0.00001, 0.0001%, 0.001%, 0.01%, 0.1, 1% and 10%) FPs using a pipette. The jars were then rotated on a cold hot dog roller under a fume hood at room temperature for 2-3 h till solvents completely evaporated and compounds homogenously covered on the inner surface of jars.

Newly emerged bee workers were prepared as described in 2016. A sugar cube was glued in the bottom of each treated jar and then a group of 10 newly-emerged worker bees were placed in the jar. The jars were covered using a piece of fabric mesh (Easy screen moustiquaire, RCR International Inc., QC, Canada) and were incubated (She Lab, Sheldon Inc., OR, USA) at 33±1 ºC and 70±2% RH for 24 h in the dark (Fig. 5). Dimethoate was used as a death control as suggested by Gough *et al*. (1994) to ensure test is working. Control treatments included applying 1 ml/jar of water, ethanol, Acetone, or Acetonitrile and a negative controls that includes solvents for each perspective tested product. Five replicates were used for each tested concentration including controls. Bee mortality was assessed to determine 24 h post-treatment.

Determination of LD_{50} tested AIs and FPs on mites using the micro-applicator assay

In 2017 this test was added to improve our testing procedure. A precision hand digital micro-applicator (Nano Infuse/Withdraw Syringe Pump KDS310, Isomass, USA) was used to apply a specific amount of tested material on mites and bees.

Micro-applicator assay for Varroa mites:

Live mites for this bioassay were collected from bee colonies with high mite infestation as described above. A digital micro-applicator fitted with a 10 µl Hamilton micro-syringe (Model 701 RN

SYR, Hamilton, USA) was used to apply tested compounds. A group of 10 Varroa mites were placed onto a piece of paper. Then each mite was topically treated with 0.15 µl of each concentration onto the dorsal shield. Treated mites were then placed directly into the 1.7 ml polypropylene centrifuge tubes containing pink-eyed pupae for feeding. Tubes were incubated at 33±1 ºC and 70±2% RH for 18 h in the dark. Mite mortality was assessed to determine 24 h post-treatment kill. Control treatments included water, ethanol, and Acetonitrile to be used for each relevant tested compound. Amitraz was used for comparison (positive control) and a no treatment control was used as a negative control.

Determination of LD_{50} tested AIs and FPs on bees using the micro-applicator assay

Micro-applicator Assay for bees:

In order to evaluate the contact toxicity (LD_{50}) of compounds to the honey bee, newly emerged bees were collected as described above. A micro-applicator fitted with a 25 µl Hamilton micro-syringe (Model 701 RN SYR, Hamilton, USA) was used to apply each tested concentration. A group of 20 newly-emerged worker bees were anesthetized by exposure to CO_2 and used for each concentration. Each bee was topically treated on the thoracic tergit with 1 µl of each concentration of AIs and FPs as described above (Fig. 6). The treated group of bees for each concentration were placed in plastic cages (5 x 5.5 x 6.5 cm) and fed sucrose solution in water (50:50) (wt: Vol). The cages were incubated at 33±1 ºC and 70±2% RH for 24 h in the dark. Bee mortality was assessed to determine 24 h post-treatment kill. Control treatments included water, Ethanol, Acetone, and Acetonitrile that were used as solvent in the experiment. Apivar (Amitraz) was used for comparison, Dimethoate (1 µl/bee contain 0.29 µg/bee, Gough et al. 1994) was used on bees for a positive death control and non-treated vials were used as a negative control.

B. Field evaluation of AIs and FPs miticides in 2016 and 2017

The main objective of this research was to assess AIs and FPs that showed potential in the laboratory test. The products needed to be effective at killing mites but safe for honey bees. To assess this, it was imperative to determine: 1) efficacy of controlling mites in bee colonies, 2) possible side effects on honey bee colonies, and 3) how they compare to currently used industry management standards (Apivar®).

The field trials were carried out at Crop Diversification Center North, Edmonton, Alberta, Canada (53.54 °N, 113.49 °W) in autumn 2016 and 2017. All bees used in this study were European honey bee (*A. mellifera*) colonies. All FPs and AIs which were used in field tests are listed in Tables 2A and 2B.

Field evaluation of AIs and FPs miticides on mites

In fall 2016, from September 29th to November 21st, single brood chamber Langstroth colonies (n=33) fitted with modified screen bottom boards (Apinovar trap) were used in field tests. Each colony was supplied with 6-8 frames of bees from highly mite infested colonies (10.24%). The alcohol wash method was used to determine the initial mean abundance of Varroa mites from each colony by sampling approximately 300 bees per hive (De Jong et al. 1982). The samples were agitated on an orbital shaker at 300 rpm for 10 min. The number of mites and bees were counted after samples were placed in a 12-mesh strainer, then rinsed under running tap water in laboratory. Varroa mites that fell through the strainer were collected in an 11L Rubbermaid® basin and counted. The bees left in the strainer were weighed to determine the number of bees in the sample. The number of bees in each sample was calculated based on the average weight of a single honey bee. This was determined by weighing three samples of 10 honey bees per sampling date. The number of Varroa mites found in the sample was then divided by the number of bees in the sample to determine the Varroa infestation level.

Pre-treatment colony evaluation

The bee population and brood area were visually assessed in all experimental colonies. To do this, colonies were scored visually and the percentage of bees covering both sides of the frame were estimated. A full frame covered with bees had approximately 2430 bees based on reported results by Burgett and Burikam (1985). Each brood frame was photographed and the area of capped brood per frame was determined using ImageJ software (Schneider et al. 2012).

To minimize genetic variation among treatments, old queens were replaced with young mated Kona queens (Kona Queen, Captain Cook, HI, USA). All queens were individually marked using numbered queen marking kits (Koniginnen Werden Gezeichnet, Germany) and introduced into the colonies. All colonies were then randomly assigned into treatments with three replicates for each treatment. Three colonies were treated with Apivar®® (Amitraz, Veto-Pharma, France) as a positive control and three colonies were left untreated as a negative control.

A substrate strip (2. 5 x 20 cm; Revel® Inc., IL, USA) was soaked in 100 ml of each dilution (1%, 5%, 10%, 15% and 20%) of FPs for 24h, however, each concentration (50 mg/strip, 100 mg/strip and 150 mg/strip) of AIs was pipetted on strips (Table 4). In order to measure the absorptive capacity of substrate strips, they were initially socked in vegetable oil for 24 h at room temperature. Each strip absorbed approximately 16.2 g of vegetable oil. FPs were directly diluted in vegetable oil, however, AIs were dissolved first in a proper solvent (e.g. acetone or acetonitrile; Sigma-Aldrich, USA), then vegetable oil in a 125 ml Erlenmeyer flask. Flasks were agitated with magnetic stirrer on a stir plate for 3 h at room temperature under the fume hood. One ml Tween80® (Fisher Scientific, Canada) was used as surfactant for FPs. Soaked substrate strips were then air dried at room temperature under the chemical hood. The prepared strips were applied to assigned bee colonies for each concentration of tested compounds. The strips were placed in between the frames in the cluster to allow bees to be exposed to the strips during the treatment period (Fig. 7).

To monitor per day mite mortality, a piece of sticky trap (30X43 cm, Contech Inc., BC, Canada) was placed on the bottom board of each hive to collect dying mites that fell through the screen. Sticky traps were removed and replaced with new ones every 1-3 days. Removed traps were covered with a thin film of plastic wrap and brought to the laboratory to count mites. Varroa mite mortality per day was calculated by dividing the number of mites found on the sticky trap by the number of days the sticky trap was in the colony.

Dead bees were collected using dead bee traps (Gary and Lorenzen 1984), which were installed in each colony 3 days prior to treatment (Fig. 8). The number of dead bees was counted every 24 h.

Field evaluation of AIs and FPs miticides in 2017

In fall 2017, field trails on single brood chamber Langstroth colonies (n=8) that were modified to three compartments were carried out. Each compartment accommodated 3 frames. An 8-mesh screen bottom board was fastened on the bottom of the box to prevent movement of bees from one compartment to the other. Overall 24 chambers were used in this experiment. Each compartment had an average of two frames of bees from highly-infested colonies (Fig. 9). The old queens were replaced with young Kona queens. The initial and final mean abundance of Varroa mites were determined from a sample of 300 bees collected from each compartment using the alcohol wash method as described above.

The pre-treatment bee population and brood area of colonies were evaluated as described above in field trails in 2016. All colonies were then randomly assigned into treatments with three replicates.

Tested products are listed in tables 2A and 2B A Substrate strip (2. 5 x 20 cm) was treated with a specific dose of products (250 mg/hive, 750 mg/hive or 1000 mg/hive). All treated substrate strips were air dried at room temperature under the chemical fume hood. Strips were then applied in the field (1 -2

strips/ colony). Sticky traps were replaced daily with fresh trap. All mites on the sticky were counted to determine daily mite mortality.

Statistical analyses:

In the lab, the variables for mite and bee mortality rates were analyzed using a mixed model ANOVA in which compounds were treated as main plots, concentrations as sub plots, glass vials or jars were experimental units (PROC MIXED, SAS Institute Inc., 2011). Non-significant factors were removed from the model. Proportions for mite mortality rate and bee mortality rate were arcsine transformed prior to analyses (Snedecor and Cochran, 1980). All data are presented as untransformed means. Where significant interactions were observed, they were partitioned using the SLICE option in LSMEANS statement and differences among treatment means were compared using Bonferroni correction (PROC MIXED, SAS Institute Inc., 2011).

The LC_{50} and LD_{50} of compounds to Varroa mites and honey bees were evaluated using PROC PROBIT (SAS Institute Inc., 2011). The percentage of mite mortality was corrected using Abbott's correction as: Corrected mortality (%) = (% mortality in treatment - % mortality in control) / (100 - % mortality in control) (Abbott 1925). The LC_{50} or LD_{50} with 95% Confidence Interval (CI) overlap was considered as non-significant different (Robertson and Preisler 1992).

Field Test Statistics.

In the Field study, the variables for cumulative daily mite and bee drop were analyzed using a mixed model ANOVA (PROC MIXED, SAS Institute Inc., 2011). These data were also analyzed by a repeated measures analysis of variance using an autoregressive heterogeneous covariance structure. A before and after control impact (BACI) design was used to assess the effects of the treatments on the mite level changes during experiments. Proportions for mite and bee drop rates were arcsine transformed prior to analyses (Snedecor and Cochran, 1980). All data are presented as untransformed means. Where

significant interactions were observed differences among treatment means were compared using Bonferroni correction (PROC MIXED, SAS Institute Inc., 2011).

Results:

Assessment of FPs on mite mortality and bees and determining their LC_{50} under lab conditions in 2016.

FPs activities against Varroa mites.

The activities of 5 formulated products: Apollo, Floramite, Shuttle, Envidor, and Forbid were tested on Varroa mites under laboratory conditions. Mitaban (active ingredient: Amitraz) was used as an active control. The mite mortality from testing FPs using the glass vial method and exposure to different concentration of compounds for 6 h was significant ($F= 11.36$; $df= 6, 18$; $p< 0.0001$) (Fig. 10). Significant results were reported for FPs tested in glass vials when mites were exposed to chemicals for 24 h ($F=36.85$; $df= 6, 118$; $p<0.0001$) (Fig. 11). Mitaban, Apollo, Floramite, Shuttle, Forbid and Envidor had significantly higher mortality than the negative control group ($p<0.05$). Comparing mite mortality among tested FPs, Mitaban had significantly higher mite mortality and Forbid had the lowest mortality ($p<0.05$). Apollo, Floramite, Shuttle and Envidor had mite mortality less than Mitaban but not significantly different from each other after 24 h exposure (Fig. 11).

Partitioning the interaction of the FPs treatment and concentrations (0.01%, 0.1%,1.0%,and 10.0%) within each tested product indicated significant differences in 6 h mite mortality within Forbid ($F= 5.10$; $df= 3, 100$; $p= 0.0025$) (Fig. 12). Partitioning the interaction of the FPs treatment and concentrations within each tested product indicated significant differences in 24 h mite mortality within Apollo ($F= 5.43$; $df= 3, 100$; $p= 0.0017$), Floramite ($F= 4.28$; $df= 3, 100$; $p= 0.0069$), Forbid ($F= 24.33$; $df= 3, 1100$; $p< 0.0001$), Shuttle ($F= 8.02$; $df= 3, 100$; $p<0.0001$), and Envidor ($F= 8.59$; $df= 3, 100$; $p< 0.0001$) (Fig. 13).

The LC_{50} for the FPs were calculated on the corrected rates of mortality after 24 h using the Abbott's formula. The relative toxicity of the five FPs to Varroa mites from highest to lowest toxicity were: Apollo> Shuttle> Envidor> Floramite> Mitaban> Forbid (Table 3).

AIs activities against Varroa mites.

When AIs of the FPs were assessed for miticidal activities against Varroa mites using the vial test, there were significant differences in mite mortalities after 6 h exposure ($F=27.19$; df= 4, 100; $p<0.0001$). Amitraz showed greater rates of Varroa mite mortality (Fig. 14). Across all AIs treatments, mite mortality rates were significantly different from each other after 24 h exposure ($F = 54.84$; df = 3, 60; $p < 0.0001$) (Fig. 15). Mean separation test showed non-significant differences between mortalities of mites exposed to Amitraz and Bifenazate. There were also no differences in mortality when mites were exposed to Acequinocyl or Bifenazate.

Partitioning the interaction of treatment and concentration within the treatment indicated significant differences in 24 h mite mortality only within Bifenazate ($F= 4.72$; df= 4, 84; $p= 0.0017$) and Spiromesifen ($F= 21.43$; df= 4, 84; $p< 0.0001$).

Highest toxicity (LC_{50}) of the AIs to Varroa mites after 24 h was found in Amitraz, Acequinocyl, Bifenazate and Spiromesifen (Table 3).

FPs activities against bees.

Mason Jars (500 mL)

There were significant differences overall between the rates of bee mortality in treatments ($F=58.00$; df= 6, 100; $p<0.0001$). The doses of Mitaban 1% and 10% increased the rate of bee death by 37% and 96%, respectively. Therefore, Mitaban data were excluded from the analyses indoor to test difference among the other 5 FPs and the control. There was no significance differences among the treatments and control ($F=2.22$; df= 5, 84; $p=0.0598$) (Fig. 16).

AIs activities against bees.

For tested AIs, bee mortality varied significantly among treatments using the Mason jar test ($F=27.09$; $df= 4, 84$; $p<0.0001$). Results showed that Amitraz had the highest bee mortality rate after 24 h exposure. When Amitraz was excluded from the analyses, Bifenazate had the highest bee mortality ($F=3.08$; $df= 3, 64$; $p=0.0143$). The rate of bee mortality in Acequinocyl and Spiromesifen was similar to the control group ($p> 0.05$) (Fig. 17).

Evaluation of Laboratory Assessments for tested FPs and their AIs.

Laboratory assessment of FPs including Apollo, Shuttle, Envidor, Floramite and Forbid showed higher Varroa mite mortality in comparison to control. The AIs of these products also showed similar results. All tested FP and AIs were safe for use on honey bees based on the laboratory test. Based on these results, FPs, Apollo, Shuttle, and Envidor and the 5 tested AIs active ingredient were selected for further testing the field.

Field Assessment of FPs and AIs on Varroa mites and honey bees.

The daily mite mortality varied significantly for FPs tested in the field ($F=10.25$; $df= 5, 50$; $p<0.0001$). High daily mite mortality was similar between Apivar and Mitaban (Amitraz) and both were significantly higher from the rest of tested FPs ($p>0.05$). The rate of mite mortality for Apollo, Envidor, Shuttle, and control was not significantly different from each other ($p>0.05$) (Table 4 and Fig. 18). There were non-significant differences overall between the average rates of daily bee mortality among treatments ($F=1.37$; $df= 5, 52$; $p=0.2507$). However, higher daily bee drop was found in 3% Mitaban and higher concentrations (> 10%) of Apollo, Envidor and Shuttle (Treatment*concentration: $F= 4.76$; $df= 12, 56$; $p<0.0001$) (Fig. 19).

In this test Apivar was used in the field to replace Amitraz. When AIs tested in the field in 2016, all AIs exhibited significant differences in mite mortality ($F=2.87$; $df= 6, 22$; $p= 0.032$) (Fig. 20). Apivar

and Spirodiclofen tested in the field showed the highest Varroa mites mortality rates, but they were not significantly different from each other. The differences in the mortality rates were insignificant for the rest of the AIs ($p>0.05$).

Lab 2017:

Measuring activities of AIs and FPs on Varroa mites and bees.

Dimethoate that was used as a control to verify the experiment is properly done, showed 100 percent mortality in bees. When solvents were tested to determine effects on bees (Fig 21 and Fig 22) and mites (Fig. 23 and 24) using the Mason jar and the micro applicator assays, the morality was not significant among treatments ($p>0.05$)

Varroa mite morality.

Glass vial assay

Mite mortality for AIs in the glass vial test was significantly different after 4h ($F= 20.82$; $df= 9, 230$; $p<0.0001$) (Fig. 25). Higher mite mortality was recorded in mites exposed to Amitraz and Fenpyroximate for 4h. Similar results were found in mite mortality in tested AIs in the glass vial test after 24h ($F= 11.93$; $df= 9, 230$; $p<0.0001$) (Fig. 26). Amitraz, Abamectin, Bifenthrin, Chlorfenapyr, Fenpyroximate and Spirotetramat had significantly high mortality of mites after 24h post-treatment.

When FPs were tested using the glass vial test, daily mite drop was significantly variable for tested products ($F= 14.76$; $df= 9, 230$; $p<0.0001$) (Fig. 27). Mortalities were not significantly different when mites exposed to Apollo, Avid, Capture, Fujimite, Pylon, and Mitaban.

Micro-applicator

Using the micro-applicator to apply specific doses, the mite mortality significantly varied among tested products ($F= 47.77$; $df= 9, 262$; $p<0.0001$) after 24 h exposure (Fig. 28). High mite mortality was observed in mites when topically treated with Amitraz, Bifenthrin, Chlorfenapyr and Fenpyroximate.

Honey Bee mortality

Mason Jar Assay

Mortality in bees was significantly higher for FPS Avid and Mitaban in comparison to the other products ($F= 59.79$; $df= 9, 467$; $p<0.0001$) (Fig. 29), and for Als Bifenthrin and Spirodiclofen ($F 26.25=$; $df= 9, 379$; $p<0.0001$) (Fig. 30) in comparison to the other products after 24 h exposure.

Micro-applicator

FPs had variable bee mortalities after 24h ($F= 10.29$; $df= 9, 381$; $p<0.0001$) (Fig. 31). Avid, Capture and Nealta killed more topically treated bees than Mitaban.

Using the micro applicator to apply accurate doses of AIs to honey bees, results showed significantly variable bee mortalities ($F= 27.22$; $df= 9, 384$; $p<0.0001$) (Fig. 32). More honey bees were killed during a 24h-period when they were topically treated with Abamectin and Bifenthrin than Amitraz.

Evaluation of Laboratory Assessments for tested FPs and their AIs.

Laboratory assessment of FPs showed 6 products had relatively higher Varroa mite mortality in comparison to control. The AIs of these products also showed similar results. Based on these results, the following FPs Avid, Pylon, Nealta, Kontos, Fujimite, and Envidor were selected for further testing the field.

Field 2017:

In fall 2017, we tested Avid, Pylon, Nealta, Kontos, Fujimite, and Envidor in the field, in addition to the AI of Envidor, Spirodiclofen. Avid (750 mg/colony) killed entire bee population in the treated colonies. Therefore, this treatment was removed from the data analyses (Table 5).

Overall a significant daily mite drop was observed for all treatments ($F= 6.02$; $df= 6, 29$; $p= 0.0003$) (Fig. 33). Fujimite and Kontos had the highest mite mortality. Analysis of variance on treatment*dose interaction showed significant interaction ($F= 11.27$; $df= 11, 24$; $p<0.0001$). The order of daily mite mortality from the highest to the lowest was Fujimite 750 mg/colony, Fujimite 250 mg/colony, Envidor 750 mg/colony, Kontos750 mg/colony, Nealta 750 mg/colony, and Spirodiclofen 750 mg/colony (Fig. 34). The treatment of infested bee colonies with Fujimite 750 mg/colony, Nealta 1000 mg/colony and Kontos 750 mg/colony decreased the mite level by 189%, 67% and 26%, respectively, during treatment period (Fig. 35).

Discussion:

Current treatments available to beekeepers for control of Varroa mites are limited and there is growing evidence that resistance is developing in the few miticides available. Inability to control Varroa mite populations not only threatens colony health, it can lead to substantial economic losses for the beekeeper. The need for new treatment options is vital for healthy bees and a thriving industry. To address this need, research conducted by Alberta Agriculture and Forestry focused on screening miticides currently registered with PMRA but not yet available to beekeepers. The ideal miticide would specifically target Varroa mites but be safe for bees during all stages of development. The compound would be required to comply with industry standards for low residuals in bee-related products (i.e. honey, wax, propolis), and be safe for the applicator. Any miticide that displays high mite mortality with no negative impacts on bees could be added to the beekeepers management strategies to control Varroa mite.

There were 11 commercial miticides that belong to 9 different chemical classes that were screened for activities on Varroa mites and bees in both the laboratory and field environment. Many of these miticides have been used to control mites in related industries such as agriculture. The miticides screened for use against Varroa mites were selected because they operate on different modes of action. This will give beekeepers alternatives for controlling this pest, while simultaneously reducing the risk of developing resistance.

The findings of this study indicate 100% mite drop in Abamectin, Bifenthrin, Chlorfenapyr, Fenpyroximate and Spirotetramat treatments after a 24 h-period when these compounds tested under laboratory conditions. This result was similar to mite mortality for Amitraz. However, testing Abamectin, Bifenthrin, and Chlorfenapyr resulted in high mortality of bees. Therefore, our results suggest that the following active ingredients: Fenpyroximate, Cyflumetofen, Spirotetramat and Spirodiclofen that belong to three chemical classes showed a greater daily mite drop and less harm to bees among the active ingredients.

A wide range of research has been carried out to screen the activities of compounds against Varroa mites and honey bees. Most researchers introduce Varroa mites into glass vials where the inner surface is homogenously coated with a miticide (Elzen *et al.* 1999, 2000; Kanga *et al.* 2010; Vandervalk 2013). Mites are left in the treated vials for 6 h then transferred into clean vials for an additional 18 h to determine mortality after 24 h. In the present study, the glass vial assay procedure was modified to include the transfer of live mites from treated glass vials to clean centrifuge tubes after the 4-hour exposure to tested compounds and mortality assessment. This was done because mites in the negative control group exhibited higher viability when transferred to centrifuge tubes compared to mites remaining in the vials for the 24-hour period of the study. This finding was applied to all treatments in the 2017 study. Further investigation is warranted to explain why the survivorship of mites has increased in centrifuge tubes. Nonetheless, this methodology has proven to be a rapid, simple and inexpensive method to screen new compounds

simultaneously for Varroacidal properties and measuring resistance of mites to the miticides under laboratory conditions.

Some key factors to consider when testing compounds for Varroacidal properties are the source population of mites and the historical exposure to applied miticides. As resistance to certain miticides becomes more prevalent in the beekeeping industry, it is necessary to screen compounds with similar modes of action to those chemicals most commonly used in beekeeping practices before beginning the experiment. Failure to do so could lead to confounding and inaccurate results. For this reason, it is strongly recommended to use the Pettis method to screen for mite resistance (Pettis et al. 1998). In this study, Amitraz, a commonly used miticide in Alberta, was tested for resistance. The results showed no resistance in mites from the lab and field trials.

Although our study did not find resistance in mites to Amitraz and Fluvalinate, sensitivity to miticides can vary dramatically between geographic regions and history of mites' exposure to miticides. For instance, Kamler et al. (2016) found higher toxicity for Amitraz in sensitive population (LC_{50}= 0.00802 µg/ml) than resistant population (LC_{50}= 0.25104 µg/ml) during 24 h period. In our study the bioassay results conclude that Amitraz has a higher lethal toxicity (LC_{50}= 0.00000152 µg/ml) using the glass vials. Conversely, studies carried out in Mexico on susceptible mites reported low LC_{50} for Amitraz of 0.23 µg/ml (Perez et al. 2000) and 0.526 µg/ml (Rodriguez-Dehaibes et al. 2005). The discrepancies in the results highlight the need for testing resistance before beginning an experiment to determine the lethal concentration of tested miticides or prescribing doses for field application.

Toxicity may be amplified by certain additives in the Formulated Products, which is another variable to consider when testing miticides. The estimated LC_{50} values for Acequinocyl and Bifenazate testing on Varroa mites were lower than Amitraz in summer 2016, whereas the LC_{50} values of the FPs of the same AIs (i.e. Shuttle and Floramite) were higher than Mitaban. This indicates that synthetic

commercial miticides increased mite mortality when compared to active ingredients under the same conditions. This may be due to the adjuvants and synergists in the formulated products. Hillesheim et al. (1996) found a significant decrease in LC_{50} values of tau-fluvalinate when Varroa mites were treated with both tau-fluvalinate and Piperonyl butoxide (PBO) (inhibitors of the cytochrome P450), while, S, S, S-tributyl phosphorotrithioate (DEF) had less synergist effects. However, Johnson et al. (2006) reported 980-fold increase in toxicity of Pyrethroids (tau-fluvalinate, cyfluthrin and lambda-cyhalothrin) mixed with PBO to honey bees. Thus, attention to effects of added adjuvants to active ingredients must be considered in evaluating toxicity against mites and bees.

Partitioning the tested concentrations by treatment showed differences in mite mortalities. Our 2017 laboratory study on Fenpyroximate showed that this miticide was more effective at higher concentrations over a short duration. These results support the reported findings of Lindber et al. (2000) and Vandervalk (2013) that found higher Varroa mite mortality as higher concentrations of different compounds were applied.

Vandervalk (2013) estimated the LC_{50} values for 5 synthetic miticides using glass vial bioassay for 24 h, suggesting significant toxicity for Shuttle more than Forbid, Floramite and Apollo, but we found more toxicity for Apollo rather than the other products. These differences in results might be due to different methods of collecting mites and incubation. Vandevalk (2013) collected mites using icing sugar method and incubated vials at room temperature. However in our experiment we used CO_2 for mite collection and all vials were incubated at 25 °C and 60% RH.

The 11 commercial miticides that belong to 9 different chemical classes were screened for activities on mites and bees. Yoshida and Fuchs (1989) indicated that Clofentezine, technical ingredient of Apollo, inhibits the Varroa mite reproduction when Varroa-infested brood cells were treated with 2.5 µg Clofentezine per cell. It also caused 66% mortality of Varroa mites when higher doses (50 µg/cell) applied.

We observed an average of 87% mortality in Varroa mites that were exposed to commercial product of Clofentezine 24 h post-treatment. However, higher concentrations of Apollo (10%) leads to more than 98% death in mites after a short period of exposure. It should be noted that chemicals like Clofentezine may be effective at controlling *V. destructor* as reported in our study, Yoshida and Fuchs (1989) and Vandervalk (2013). However, Yoshida and Fuchs (1989) found that this chemical also affects the metamorphosis of honey bee larvae. Therefore, despite its efficacy on Varroa mites, it is not recommended to be used in honey bee colonies.

Despite glass vial bioassays being a widely accepted method to determine toxicity of chemicals, the amount of miticide contracted by mites and bees is unknown. Consequently, researchers are not able to determine the LD_{50}. To overcome this obstacle, we did preliminary research using a precision micro-applicator to apply a measured dose with specific concentrations of AIs and FPs on Varroa mites and bees. Thus, we were able to adopt this system of using the micro-application in applying a precise dose to mites and bees that enable us to determine the LD_{50} for tested compound as presented in Table 6.

In numerous studies, honey bee workers were exposed to pesticides through a variety of methods including feeding (Lauriro et al. 2011), surface contact in Petri dishes (Lindberg *et al.* 2000) and topical contact (Mao et al. 2011; Johnson et al. 2013). The 2016 Mason jar experiment exposed worker bees to chemicals using coated plastic strips in Mason jars, which mimics the strip used for Apistan®, Apivar®® or Bayvarol®. In 2017 we used Mason jars that have the inner coated with miticides. These methods will be further evaluated and compared to the field test using bee colonies. If a correlation was found, a test will be recommend for use in screening miticides to reduce cost of testing.

The 2016 bioassay experiments in Mason jars confirmed a significantly greater bee mortality in treated bees with high doses of Bifenazate, Amitraz and Mitaban. For example when bees were exposed to 0.1% and 1% Amitraz in the Mason jar test in 2016, each bee was exposed to approximately 17.22 µg/cm^2

and 72.24 µg/cm^2 of Amitraz, respectively. Consequently, bee mortality was 10.39±1.28% and 9.53±1.28% for bees exposed to Amitraz concentration of 0.1 and 1%, respectively. Currently Varroa infested colonies are treated with Apivar®®, a plastic strip impregnated with 3.33% Amitraz, which equates to 50 mg technical ingredients per strip (Veto-Pharma, Mauritius, France) for a 6-8 week treatment period. Typically a single brood chamber of honey bee colonies contains approximately 20,000 individual bees that would be treated with 2 strips of Apivar® in autumn. In this case, bees would be exposed to at least 5 µg/cm^2 of the technical ingredient of Amitraz during treatment period. To explain reported high bee mortality in our experiment, bees were exposed to a larger amount of Amitraz for a short time in comparison to field doses when bees exposed to Apivar strips in bee colonies. Therefore a higher bee mortality is expected in our results. Additionally, in 2017 trials, similar results of high bee mortalities were reported when Abamectin, Bifenthrin and Chlorfenapyr when tested in the laboratory. Overall these results suggest that if tested compounds are not selective miticide that kill mites and safe for bees. Therefore, a dose response test must be also done to find a dose that can kill mites but it is safe for the bees.

The field assessments of selected compounds showed in 2016 similar results to what was reported in the laboratory testing in 2016 and 2017. High bee mortality was found when Bifenazate and Spiromesifen were applied. Results of Varroa mite mortality and bee mortality under field conditions showed that products of Tetronic acids, Quinazolines and Benzoylacetonitriles have potential to decease the mite population levels in honey bee colonies when bees were treated with 750 mg/ colony. Therefore, future investigation is needed to find a proper dose for each product to control Varroa mites, long term effects on bees, brood production, honey production and residues in honey and wax overtime.

In conclusion, this research has established bioassay procedures for screening potential compounds for Varroa control and bee safety. These assays include testing activities of compounds under laboratory conditions for mite and bee morality using the vial test. The microapplicator was adopted to apply specific doses to Varroa mites as well as bees. Overall this research project has identified at least 4 AIs belong to

3 chemical classes different from currently used miticides for Varroa. These compounds can be potentially developed to be used in the field by beekeepers. Thus, it will add significant economic value to beekeeping and agriculture in Canada. It will also enable beekeepers to have effective Varroa control system and improve bee health.

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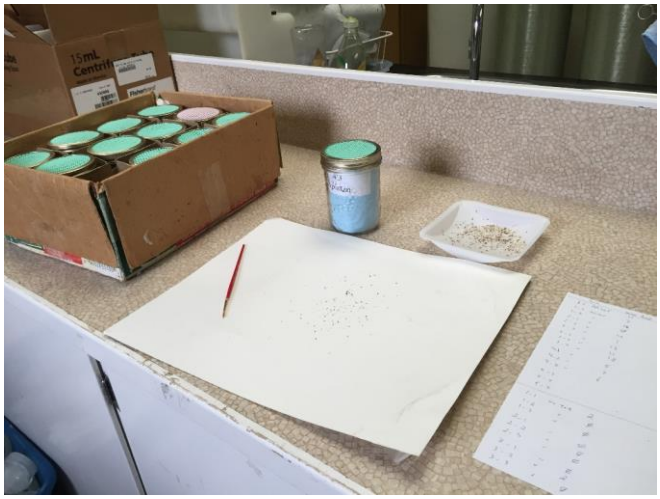

Fig. 1. Resistance test of Varroa mites to Apivar® (Amitraz) abd Apistan® (Fluvalinate):

Fig. 2. Treated scintillation glass vials are rotated on a cold hot dog roller under the fume hood at room temperature for 2-3 h till solvents completely evaporated and compounds are homogenously speared on the inner surface of vials.

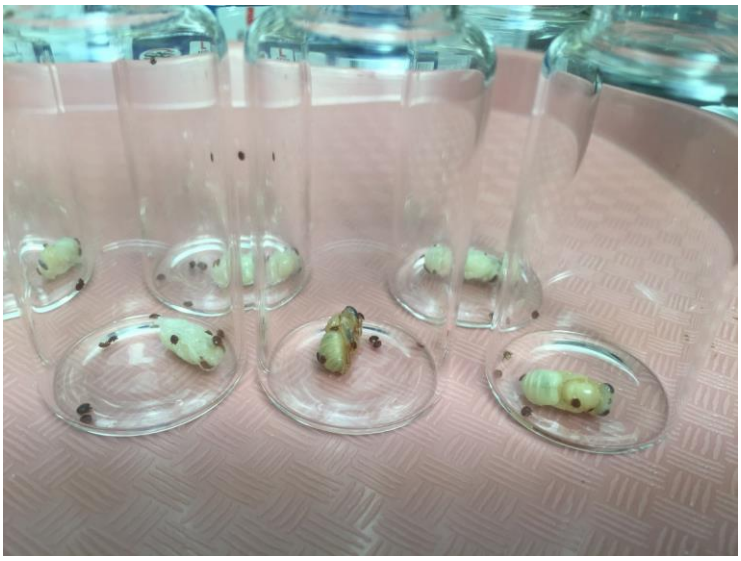

Fig. 3. A group of 8-10 mite are introduced into the treated scintillation glass vials and feed pink-eyed pupae.

Fig. 4. A group of newly-emerged worker bees are exposed to tested compounds in The Mason jar Assay (2016).

Fig. 5. A group of newly-emerged worker bees are exposed to tested compounds in 200 g- Mason jars assay ((2017).

Fig. 6. A newly-emerged worker bee is topically treated 1 µl of tested compound using Hamilton micro syringe in a precision micro-applicator.

Fig. 7. Placing a treated substrate strip between frames within the experimental hive (2016).

Fig. 8. Dead bees are removed from the experimental hives using Gary dead bee trap (2016).

Fig. 9. The experimental hive with three compartments (2017).

Table 1. List of tested compounds and their mode of action.

Formulated Product	Active ingredient	Concentration	Class	Mode of Action
Apollo	Clofentezine	50%	Tetrazines	Growth inhibitor
Shuttle	Acequinocyl	15.8%	Quinolines	Inhibits the respiration of mitochondria
Envidor	Spirodiclofen	24%	Tetronic acids	Inhibitor of acetyl-CoA carboxylase
Floramite	Bifenazate	22.6%	Carbazates	Inhibits the respiration of mitochondria
Forbid	Spiromesifen	24%	Tetronic acids	Inhibitor of lipid biosynthesis
Mitaban	Amitraz	19.9%	Formamidines	Octopamine receptor agonist
Avid	Abamectin	1.98%	Avermectins	GABA agonist
Kontos	Spirotetramat	22.4%	Tetronic acids	Inhibitor of lipid biosynthesis
Capture	Bifenthrin	24%	Pyrethroids	Effect on voltage-sensitive ion channels
Pylon	Chlorfenapyr	24%	Pyrroles	Effect on oxidative phosphorylation
Fujimite	Fenpyroximate	5%	Quinazoline	Inhibitor of ATP synthase
Nealta	Cyflumetofen	20%	Benzoylacetonitrile	Inhibitor of mitochondria complex II

Table 2A. List of tested formulated compounds, providers and testing time (X).

Product	Provider	2016		2017	
		Lab test	Field test	Lab test	Field test
Apollo	Adama, NC, USA	X	X	X	-
Shuttle/ Kanemite	Arysta life Science, NC, USA	X	X	X	-
Envidor	Bayer, Canada	X	X	X	X
Floramite	Chemtura, ON, Canada	X	-	-	-
Forbid	Bayer, Canada	X	-	-	-
Mitaban	Zoetis, MI, USA	X	X	X	-
Avid	Syngenta, ON, Canada	-	-	X	X
Kontos	Bayer, Canada	-	-	X	X
Capture	FMC, Canada	-	-	X	X
Pylon	BASF, NC, USA	-	-	X	X
Fujimite	Engage Agro, ON, Canada	-	-	X	X
Nealta	BASF, NC, USA	-	-	X	X

Table 2B. List of tested active ingredients, providers and testing time (X)

Product	**Provider**	**2016**		2017	
		Lab Test	**Field Test**	**Lab Test**	**Field Test**
Clofentezine	Sigma-Aldrich, Canada	-	**X**	**X**	-
Acequinocyl	Sigma-Aldrich, Canada	**X**	**X**	**X**	-
Spirodiclofen	Sigma-Aldrich, Canada	-	**X**	**X**	**X**
Bifenazate	Sigma-Aldrich, Canada	**X**	**X**	-	-
Spiromesifen	Sigma-Aldrich, Canada	**X**	**X**	-	-
Amitraz	Sigma-Aldrich, Canada	**X**	-	**X**	-
Abamectin	Sigma-Aldrich, Canada	-	-	**X**	-
Spirotetramat	Sigma-Aldrich, Canada	-	-	**X**	-
Bifenthrin	Sigma-Aldrich, Canada	-	-	**X**	-
Chlorfenapyr	Sigma-Aldrich, Canada	-	-	**X**	-
Fenpyroximate	Sigma-Aldrich, Canada	-	-	**X**	-
Cyflumetofen	Cedarlane, ON, Canada	-	-	**X**	-

Table 3. Lethal concentration values (LC_{50}) for 24 h responses of *V. destructor* mites to tested acaricides in present study (2016-2017) and Vandervalk (2013).

Product	2016-2017 Study		2013 Vandervalk	
	LC_{50}	95%CI	LC_{50}	95%CI
Apollo	3.13E-35	1.51E-5 - 0.0162	4.268	3.469-5.022
Floramite	0.00661	7.18E-4 - 0.02671	6.267	4.949-7.705
Forbid	0.00944	-	4.63	2.669-7.904
Shuttle/Kanemite	1.12E-19	4.46E-5 - 0.01897	1.341	0.936-1.877
Envidor	4.54E-41	2.73 E-4 - 0.01746	-	-
Mitaban	0.00887	-	-	-
Avid	1.76E-06	3.8E-9 - 3.64E-5	-	-
Kontos	7.17E-25	-	-	-
Capture	9.12E-06	7.57E-06	-	-
Pylon	1.93E-07	2.81E-25	-	-
Fujimite	3.23E-06	2.36E-08	-	-
Nealta	3.06E+70	-	-	-
Bifenazate	2.06E-06	3.99E-12 - 0.000142	-	-
Spiromesifen	7.03E-06	3.6E-8 - 0.000143	-	-
Acequinocyl	9.52E+23	2.58E-13 - 0.000106	-	-
Clofentezine	4.55E+23	-	-	-
Spirodiclofen	5.81E-61	-	-	-
Amitraz	1.52E-10	8.18E-70 – 1.5E-7	-	-
Abamectin	1.45261	-	-	-
Spirotetramat	1.78E+22	-	-	-
Bifenthrin	5.28E-02	-	-	-
Chlorfenapyr	3.03E-06	1.23E-12 – 2.76E-5	-	-
Fenpyroximate	9.91E-07	-	-	-
Cyflumetofen	3691	1.06781- 1.45E+29	-	-

Table 4. Evaluation of the efficacy of Formulate Products (FP) and their Active Ingredients (AI) of against Varroa mites and side effects on bees under field condition in 2016.

Treatment	Average daily mite mortality (±SE)	Average daily bee mortality (±SE)	Concentration or dose	AI mg/colony
Formulate Products				
Apollo	69.69±31.77	104.67±155	1%	0.324
			5%	1.62
			10%	3.24
			15%	4.86
			20%	6.48
Envidor	59.01±31.77	143.00±15.55	1%	0.324
			5%	1.62
			10%	3.24
			15%	4.86
			20%	6.48
Shuttle	54.25±31.77	180.50±19.04	1%	0.324
			5%	1.62
			10%	3.24
			15%	4.86
Mitaban	232.21±55.03	110.00±26.92	3%	0.972
Active Ingredients				
Acequinocyl	67.96±67.77	64.67±17.80	50 mg	50
			100 mg	100
			150 mg	150
Bifenazate	59.13±67.77	68.33±17.80	50 mg	50
			100 mg	100
			150 mg	150
Clofentezine	30.47±83.00	29.50±17.80	50 mg	50
			100 mg	100
			150 mg	150
Spirodiclofen	116.67±67.77	30.33±17.80	50 mg	50
			100 mg	100
			150 mg	150
Spiromesifen	50.02±67.77	64.33±17.80	50 mg	50
			100 mg	100
			150 mg	150
Apivar	253.96±39.13	44.56±10.28	3.33%	1000
Control	69.45±47.92	26.83±12.60	-	-

Table 5. Evaluation of the efficacy of Formulate Products (FP) and their Active Ingredients (AI) against Varroa mites and side effects on bees under field condition in 2017.

Treatment	AI mg/colony	Average daily mite mortality (±SE)
Formulate Products		
Envidor	250	0.01637±0.01906
	750	0.06753±0.01906
Fujimite	250	0.0863±0.01906
	750	0.2086±0.01906
Kontos	750	0.1131±0.01906
Nealta	250	0.03597±0.01906
	750	0.0684±0.01906
	1000	0.02067±0.01906
Pylon	750	0.0188±0.01906
Active Ingredient		
Spirodiclofen	250	0.02593±0.01906
	750	0.09337±0.01906
Control	0	0.01367±0.02257

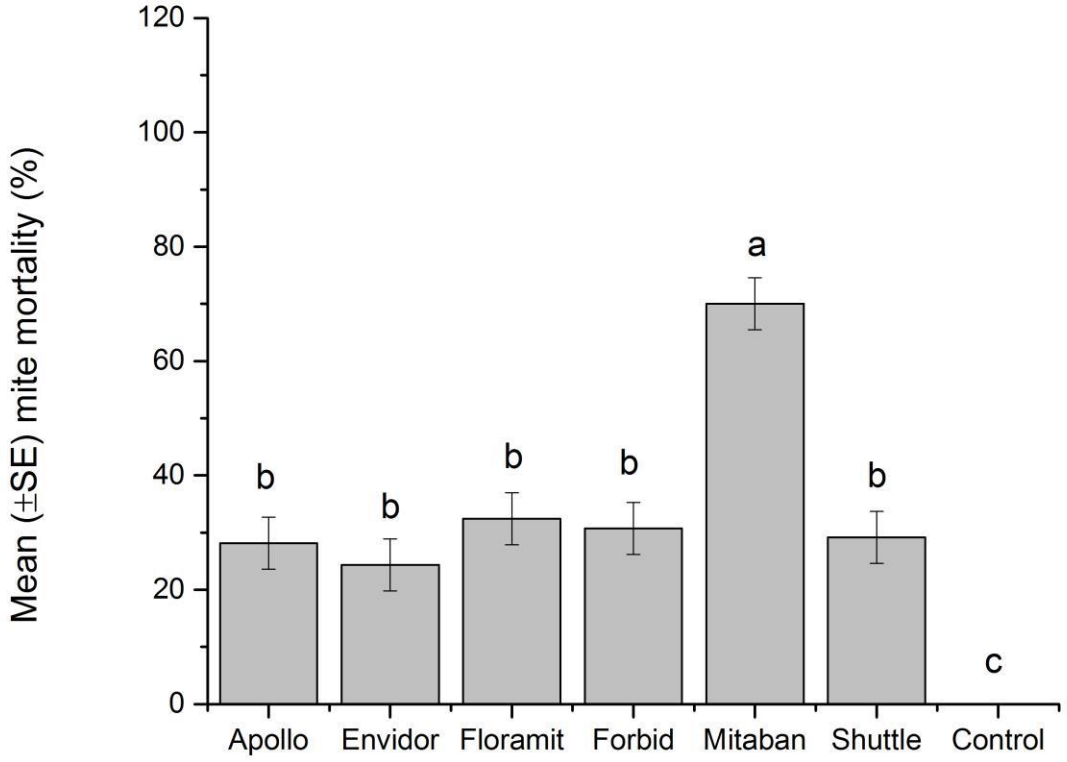

Fig. 10. Mean (±SE) percentage mite mortality during 6 h exposed to tested formulated products in glass vials under laboratory conditions (2016). Means with the same letter among treatments are not significantly different ($p<0.05$).

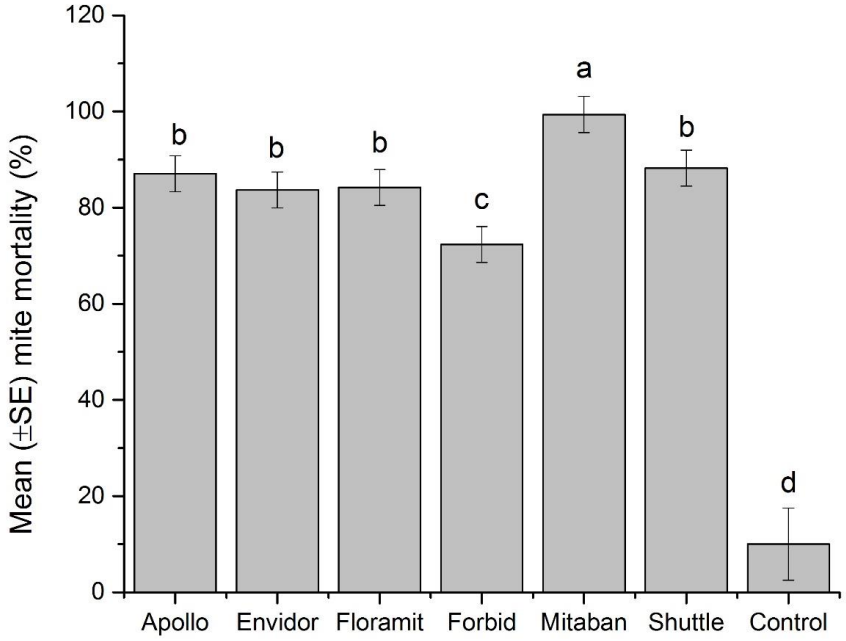

Fig. 11. Mean (±SE) percentage mite mortality during 24 h exposed to tested formulated products in glass vials under laboratory conditions (2016). Means with the same letter among treatments are not significantly different ($p<0.05$).

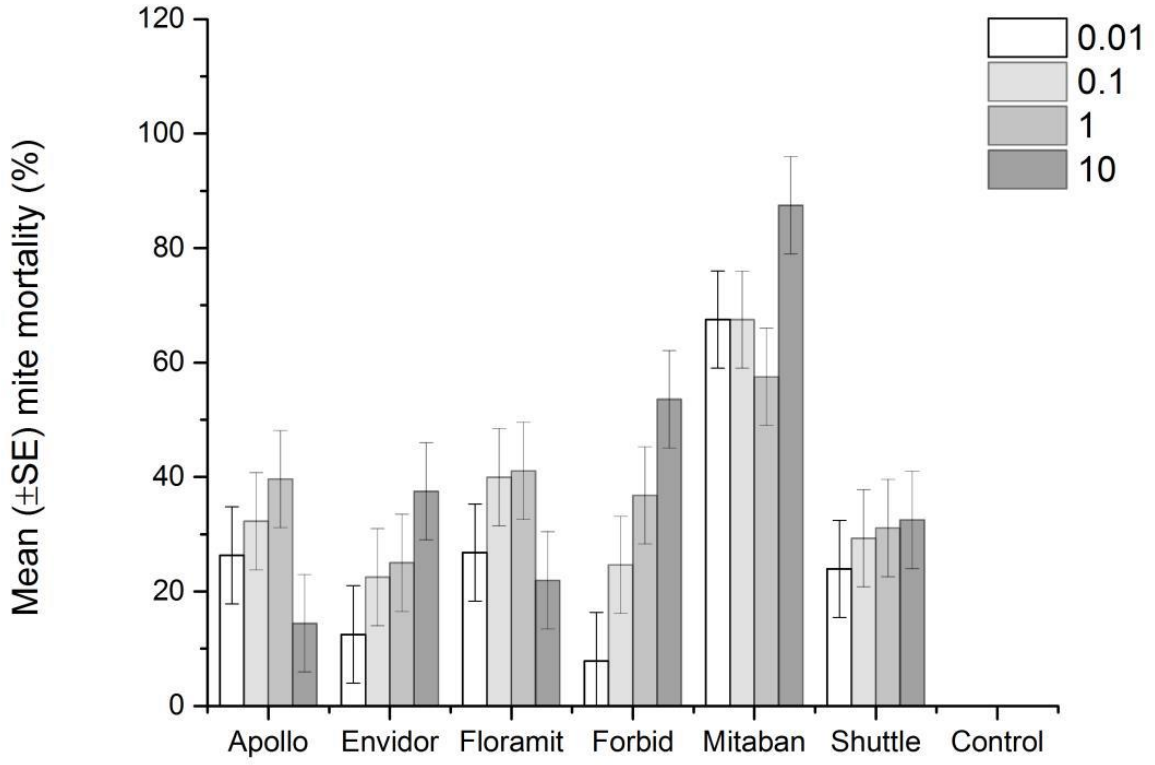

Fig. 12. Mean (±SE) percentage mite mortality during 6 h exposed to different concentrations of tested formulated products in glass vials under laboratory conditions (2016).

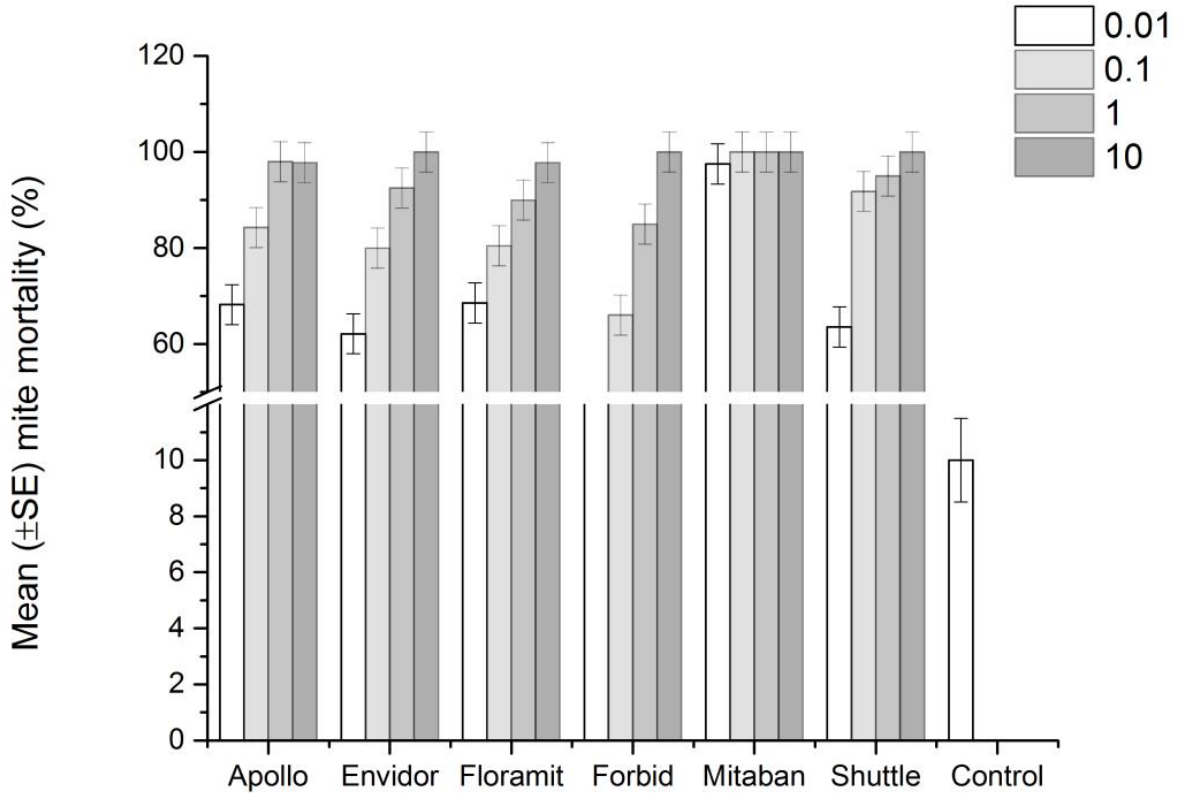

Fig. 13. Mean (±SE) percentage mite mortality during 24 h exposed to different concentrations of tested formulated products in glass vials under laboratory conditions (2016).

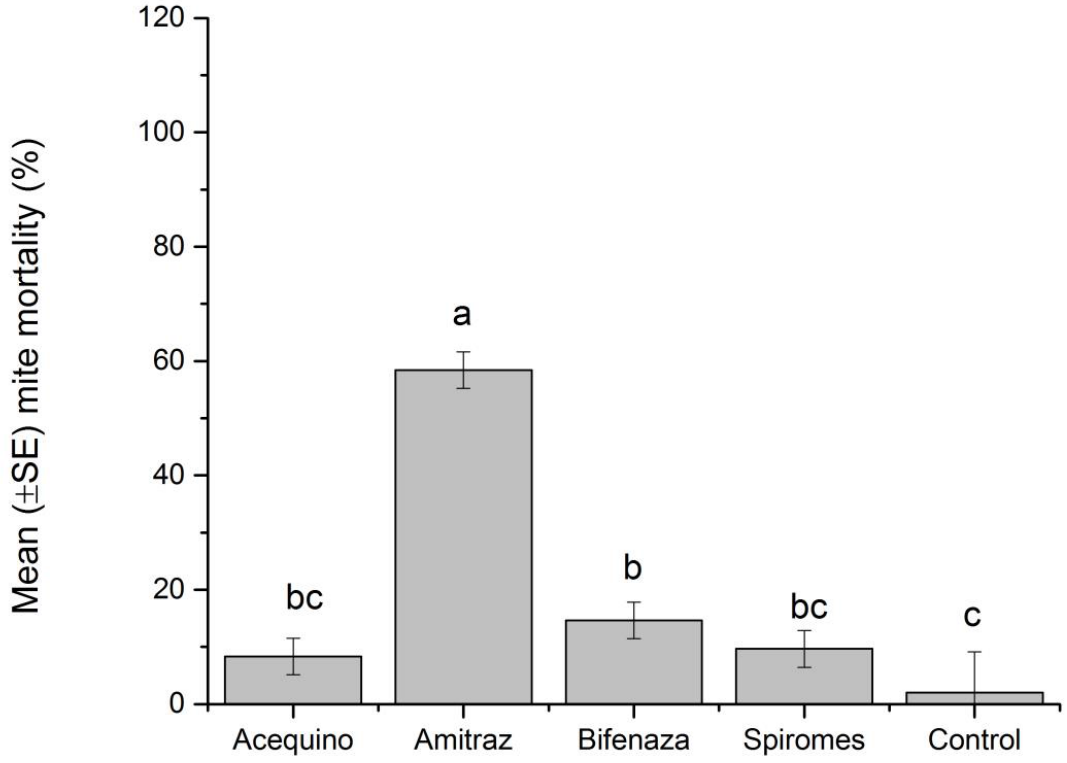

Fig. 14. Mean (±SE) percentage mite mortality during 6 h exposed to tested active ingredients in glass vials under laboratory conditions (2016). Means with the same letter among treatments are not significantly different ($p<0.05$).

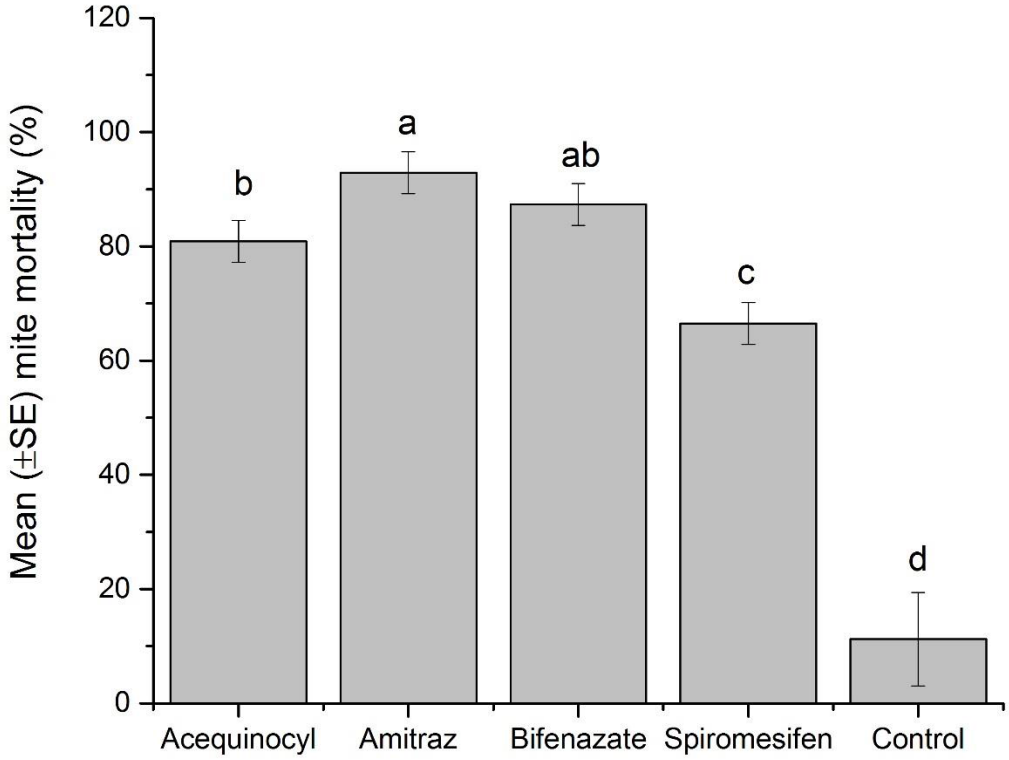

Fig. 15. Mean (±SE) percentage mite mortality during 24 h exposed to tested active ingredients in glass vials under laboratory conditions (2016). Means with the same letter among treatments are not significantly different ($p<0.05$).

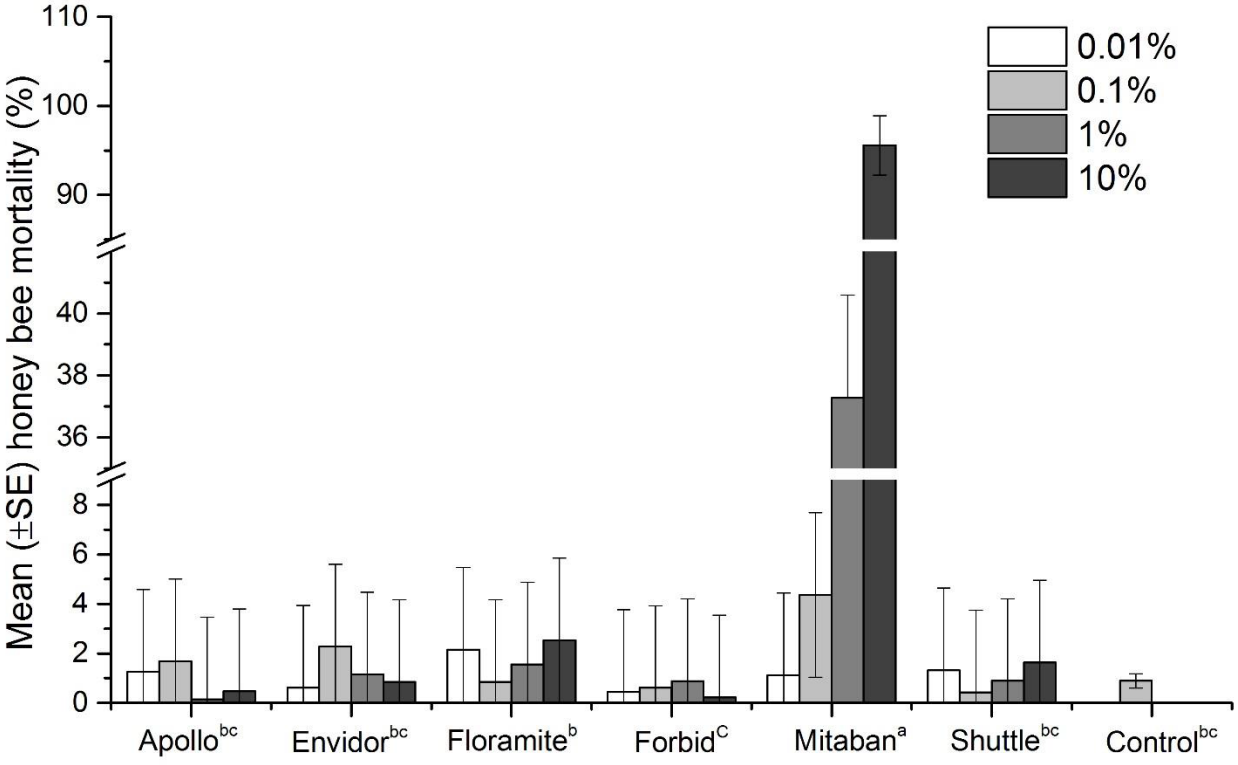

Fig. 16. Mean (±SE) percentage bee mortality during 24 h exposed to different concentrations of tested formulated products in Mason jars under laboratory conditions (2016). Means with the same letter among treatments are not significantly different ($p<0.05$).

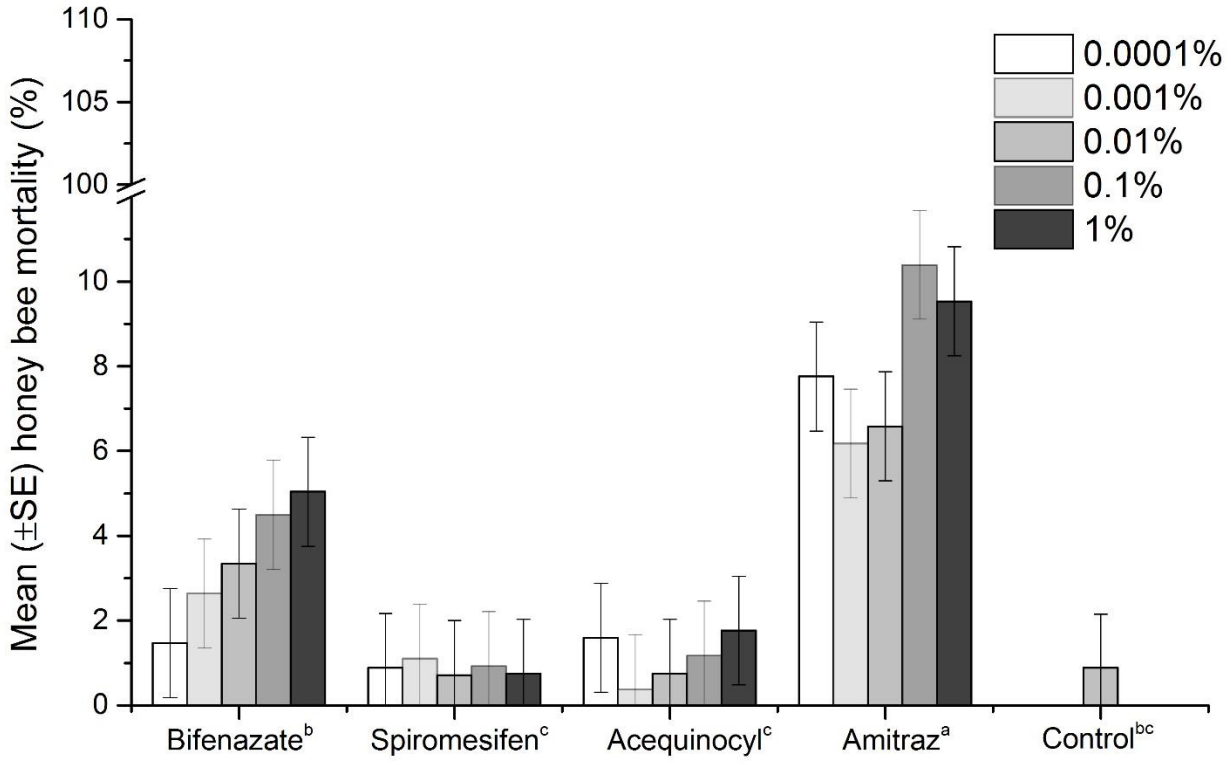

Fig. 17. Mean (±SE) percentage bee mortality during 24 h exposed to different concentrations of tested active ingredients in Mason jars under laboratory conditions (2016). Means with the same letter among treatments are not significantly different ($p<0.05$).

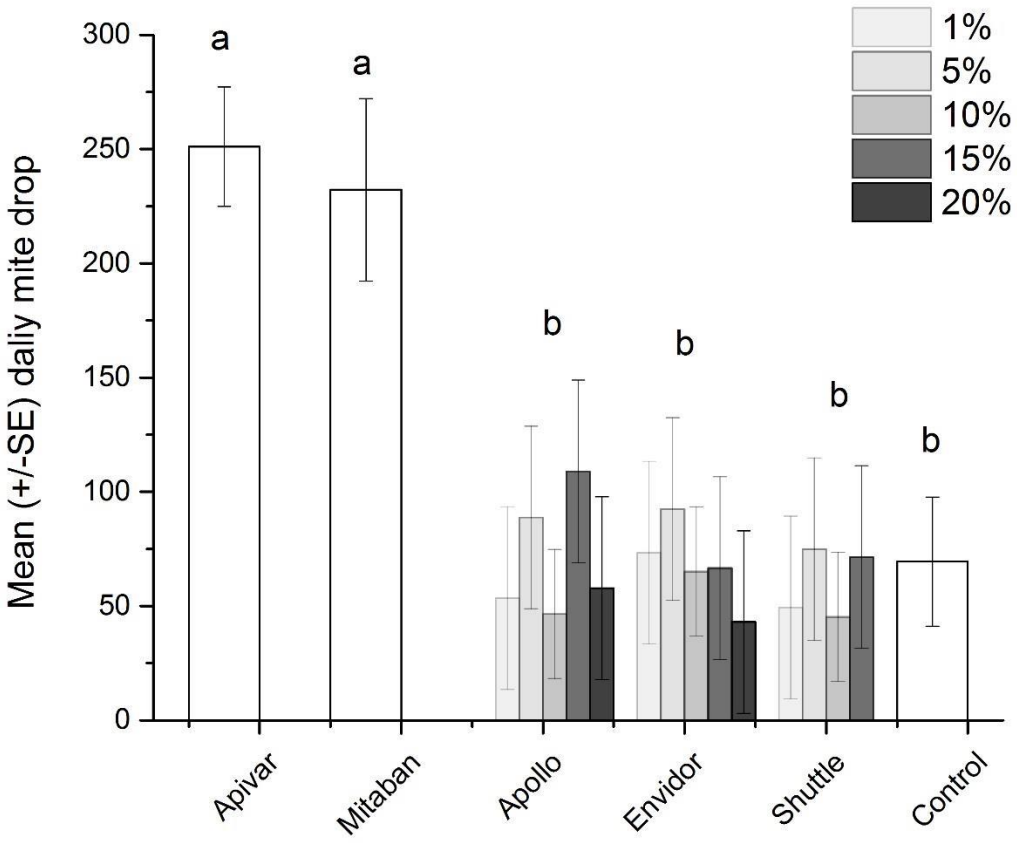

Fig. 18. Mean (±SE) daily Varroa mites drop exposed to different concentrations of tested formulated products (Apollo®, Envidor® and Shuttle®) in honey bee colonies. Apivar® and Mitaban® were used as positive control (2016). Means with the same letter among treatments are not significantly different ($p<0.05$).

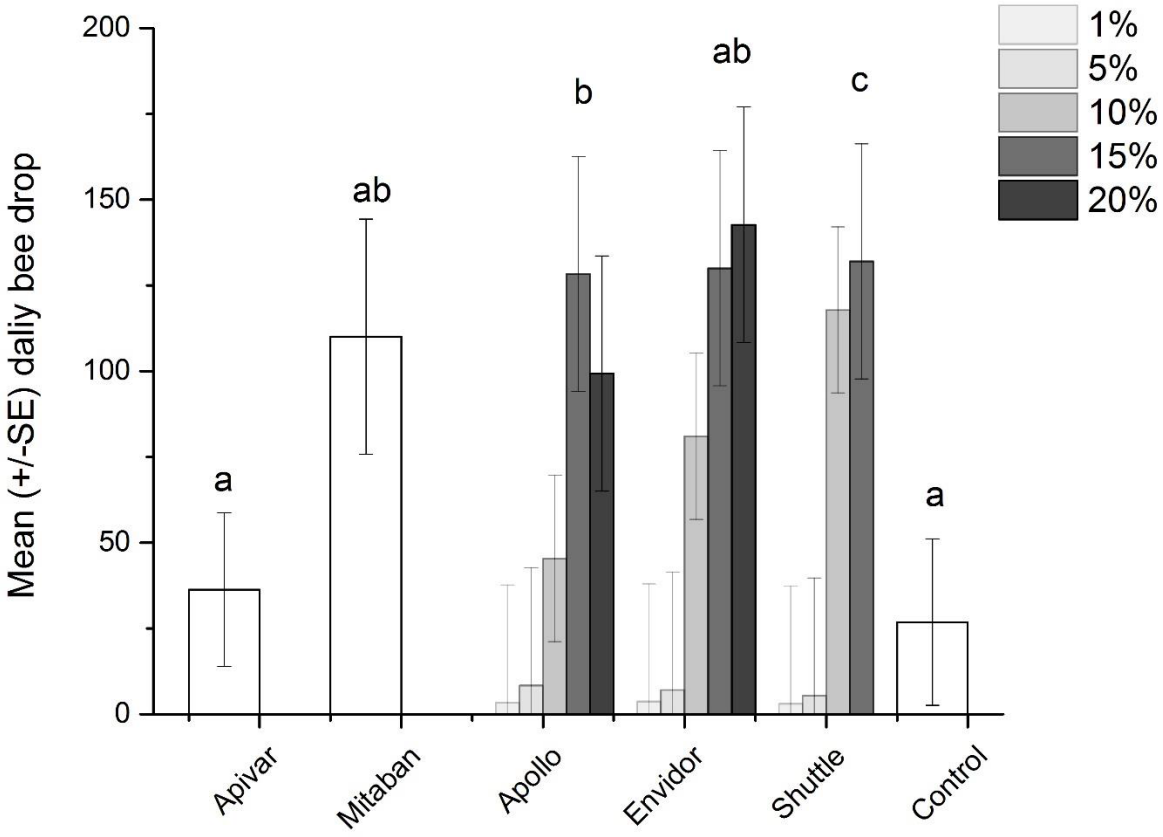

Fig. 19. Mean (±SE) daily bee drop exposed to different concentrations of tested formulated products (Apollo®, Envidor® and Shuttle®) in honey bee colonies. Apivar® and Mitaban® were used as positive control (2016). Means with the same letter among treatments are not significantly different ($p<0.05$).

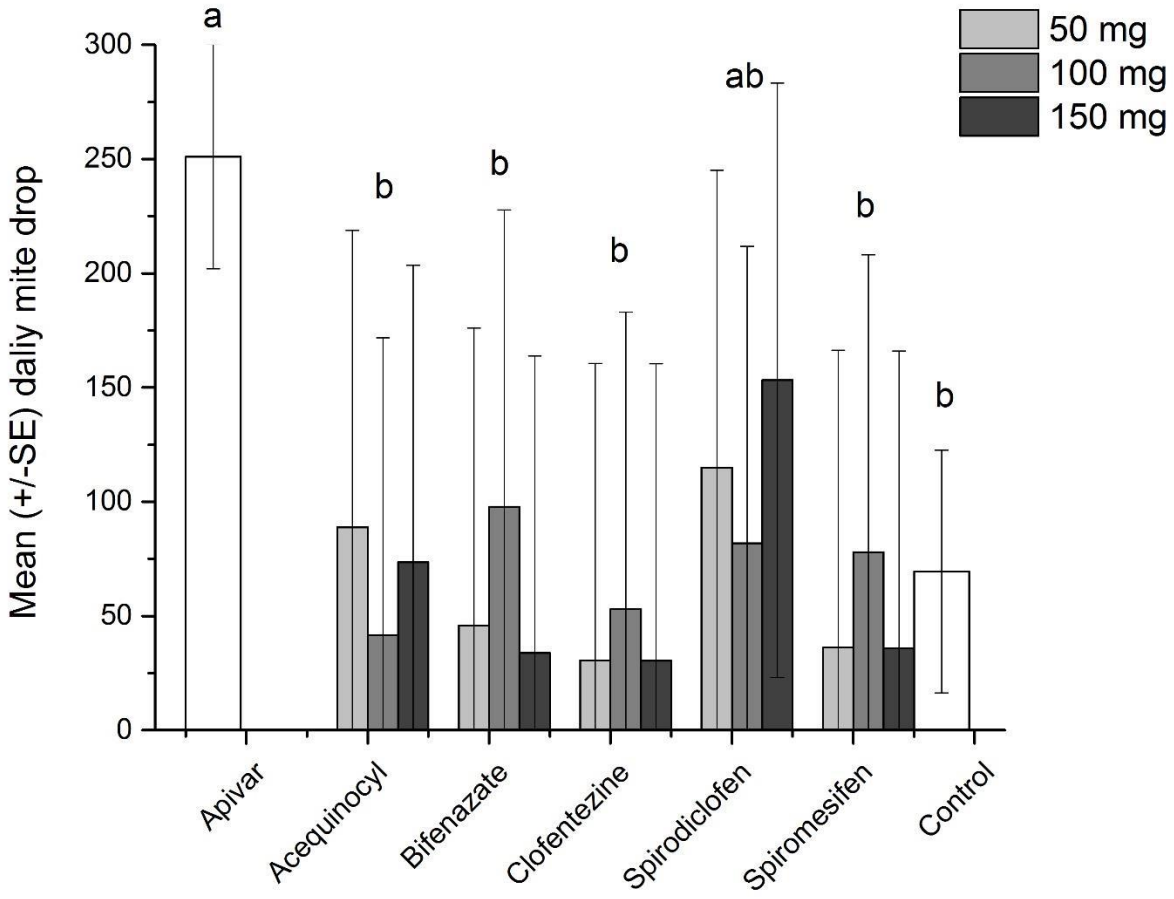

Fig. 20. Mean (±SE) daily Varroa mites drop exposed to different doses (mg/strip) of tested active ingredients (Acequinocyl PESTANAL®, Bifenazate PESTANAL®, Clofentezine PESTANAL®, Spirodiclofen PESTANAL® and Spiromesifen PESTANAL®) in honey bee colonies. Apivar® was used as positive control (2016). Means with the same letter among treatments are not significantly different ($p<0.05$).

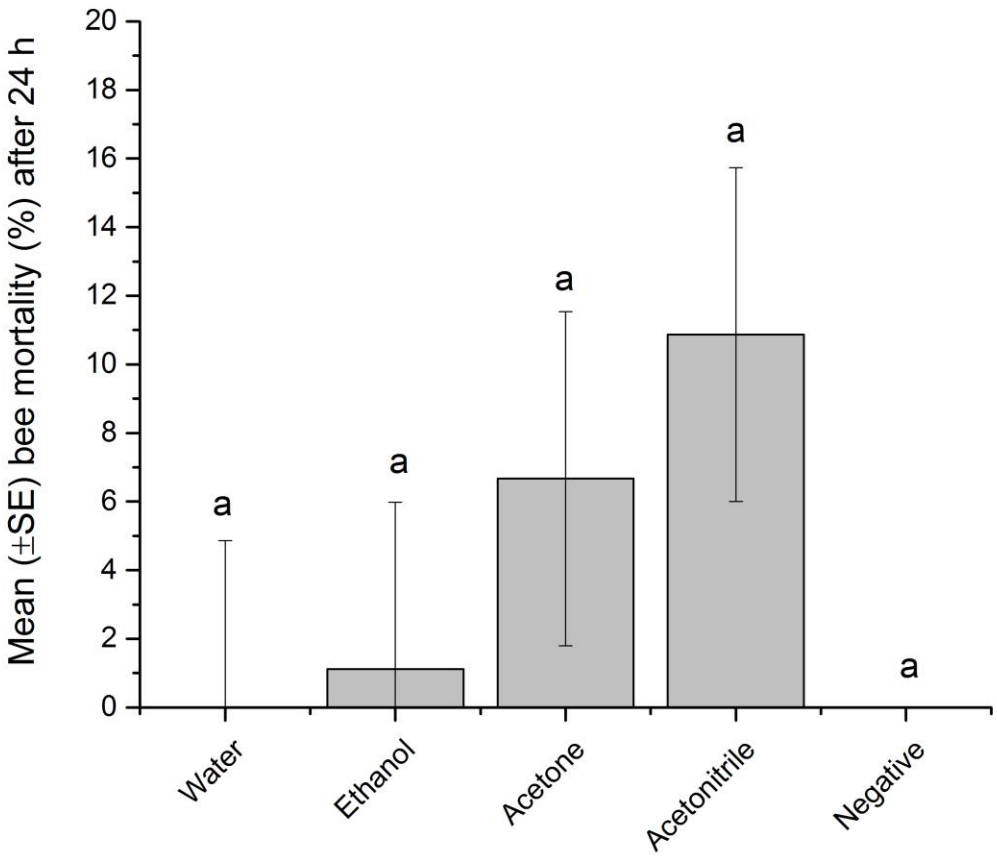

Fig. 21. Mean (±SE) daily bee drop in control group after 24 h in micro applicator assay (2017). Means with the same letter among treatments are not significantly different (p<0.05).

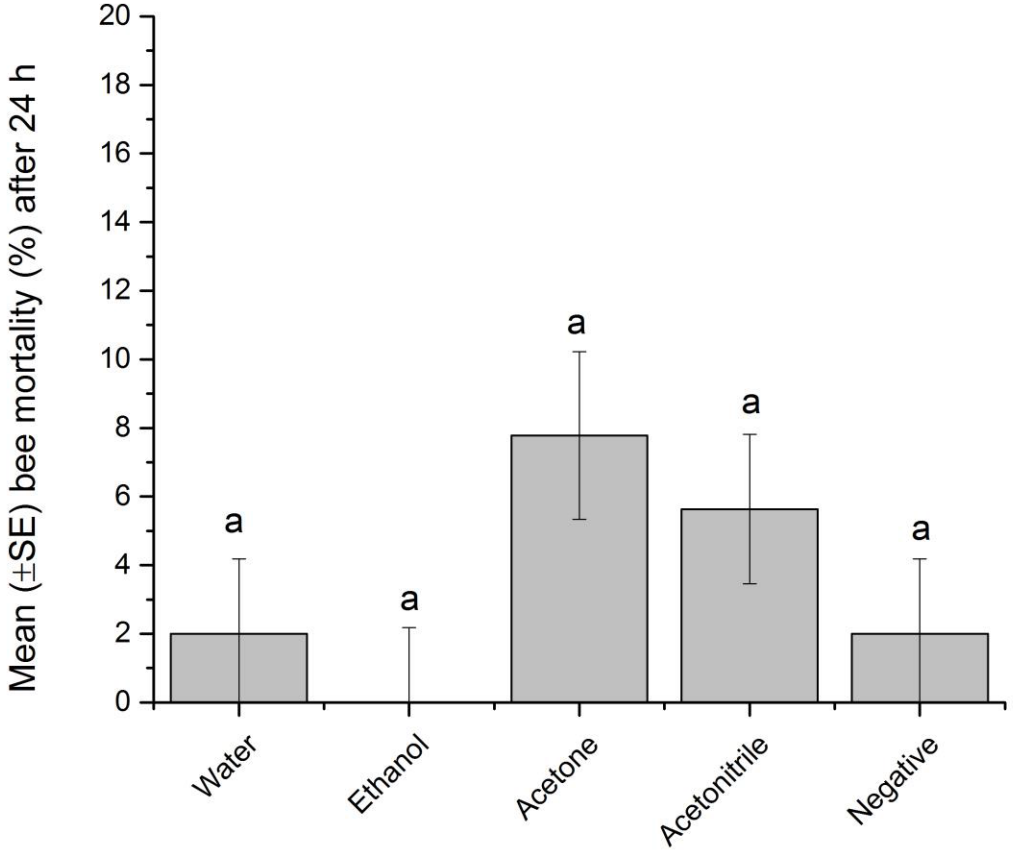

Fig. 22. Mean (±SE) daily bee drop in control group after 24 h in Mason jar assay (2017). Means with the same letter among treatments are not significantly different (p<0.05).

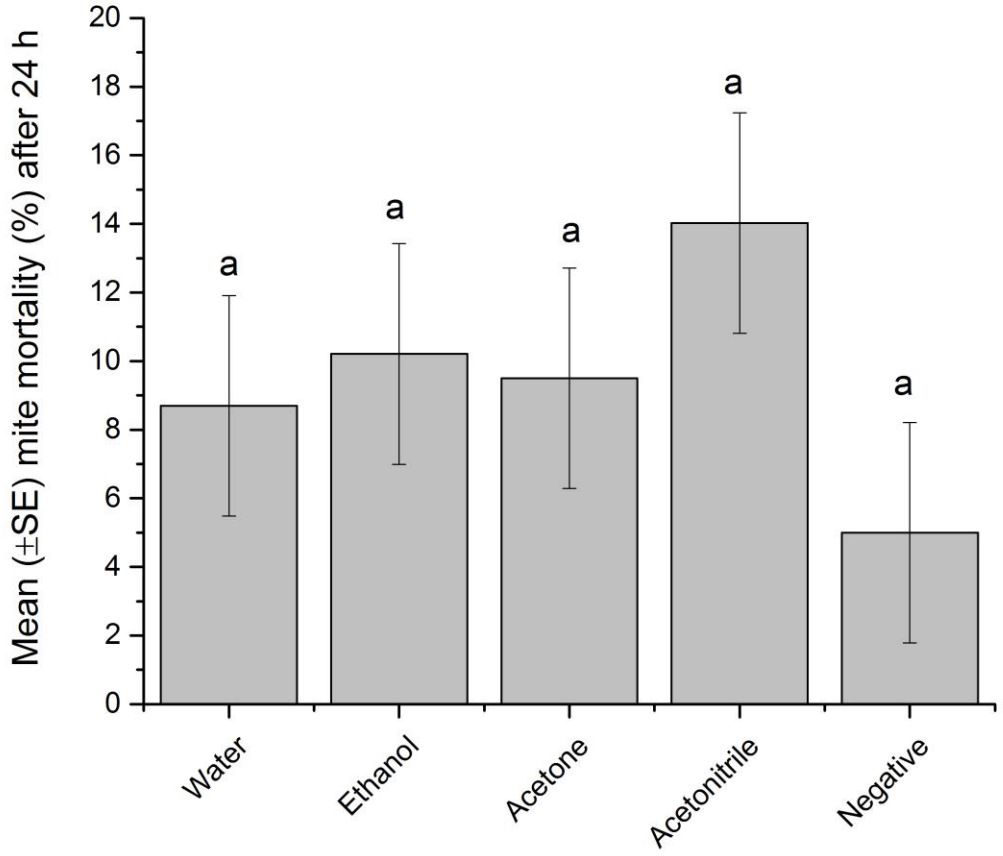

Fig. 23. Mean (±SE) daily mite drop in control group after 24 h in glass vial assay (2017). Means with the same letter among treatments are not significantly different (p<0.05).

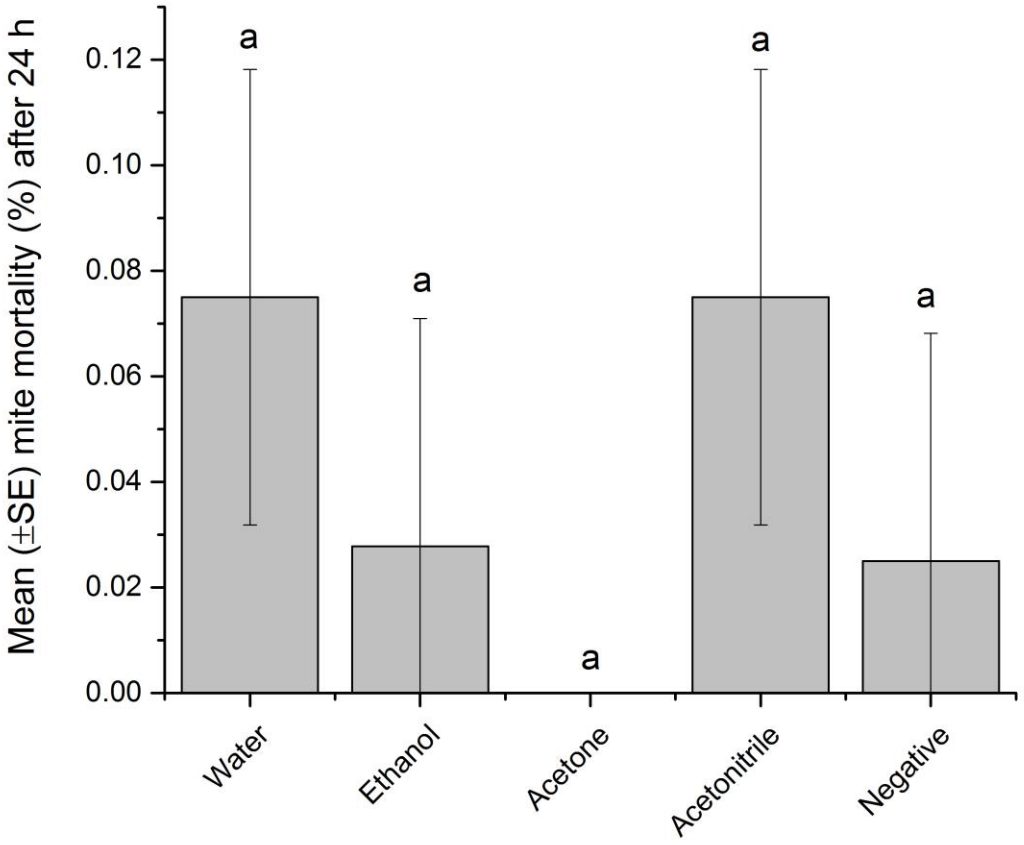

Fig. 24. Mean (±SE) daily mite drop in control group after 24 h in micro applicator assay (2017). Means with the same letter among treatments are not significantly different (p<0.05).

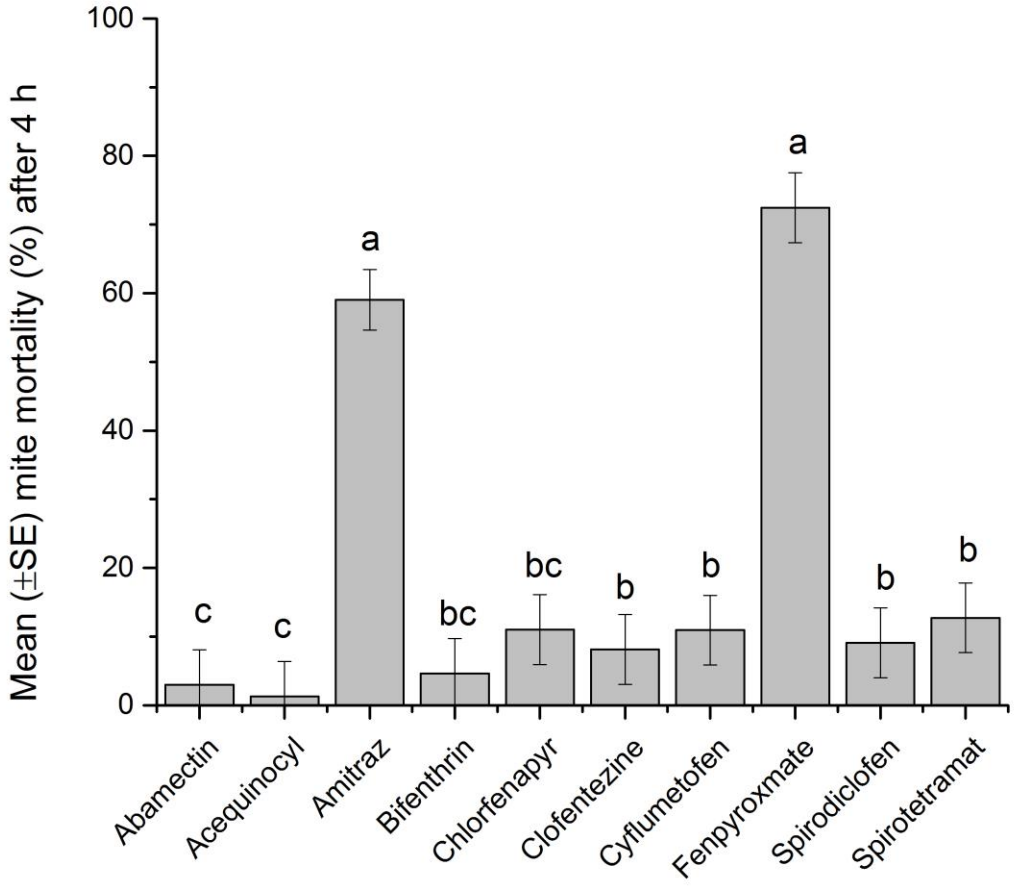

Fig. 25. Mean (±SE) daily mite drop exposed to active ingredients after 4 h in glass vial assay (2017). Means with the same letter among treatments are not significantly different (p<0.05).

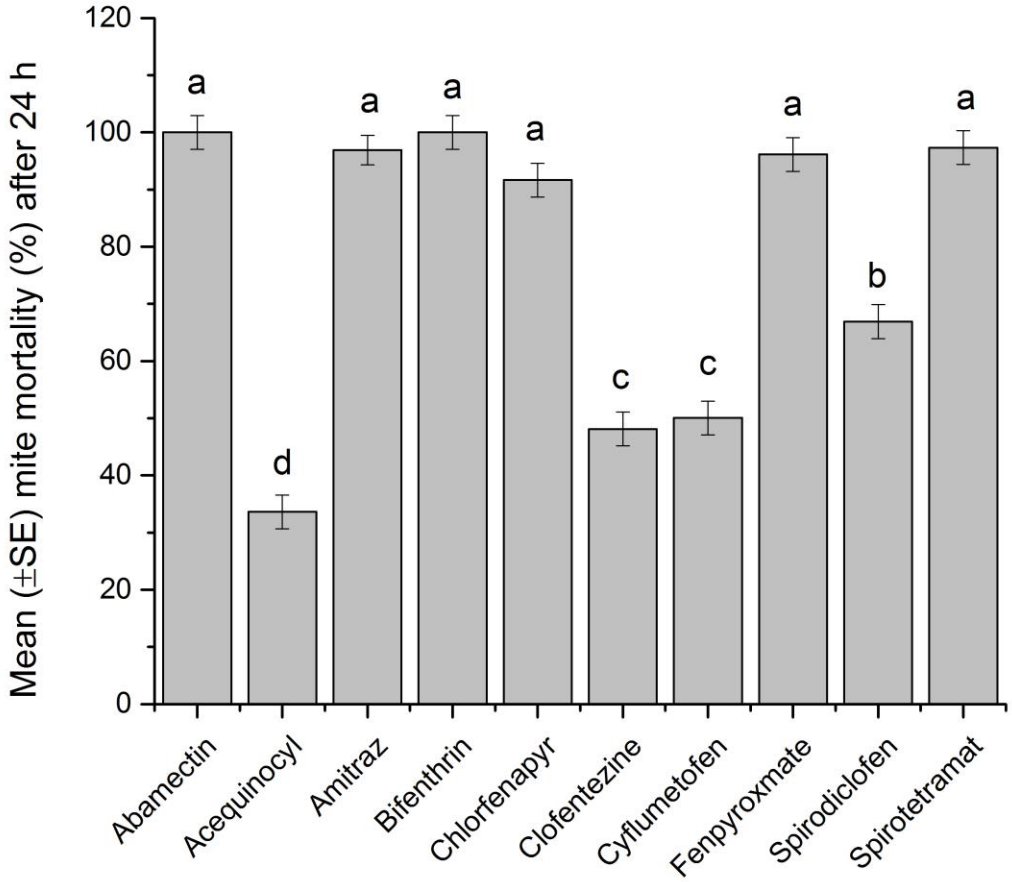

Fig. 26. Mean (±SE) daily mite drop exposed to tested active ingredients after 24 h in glass vial assay (2017). Means with the same letter among treatments are not significantly different (p<0.05).

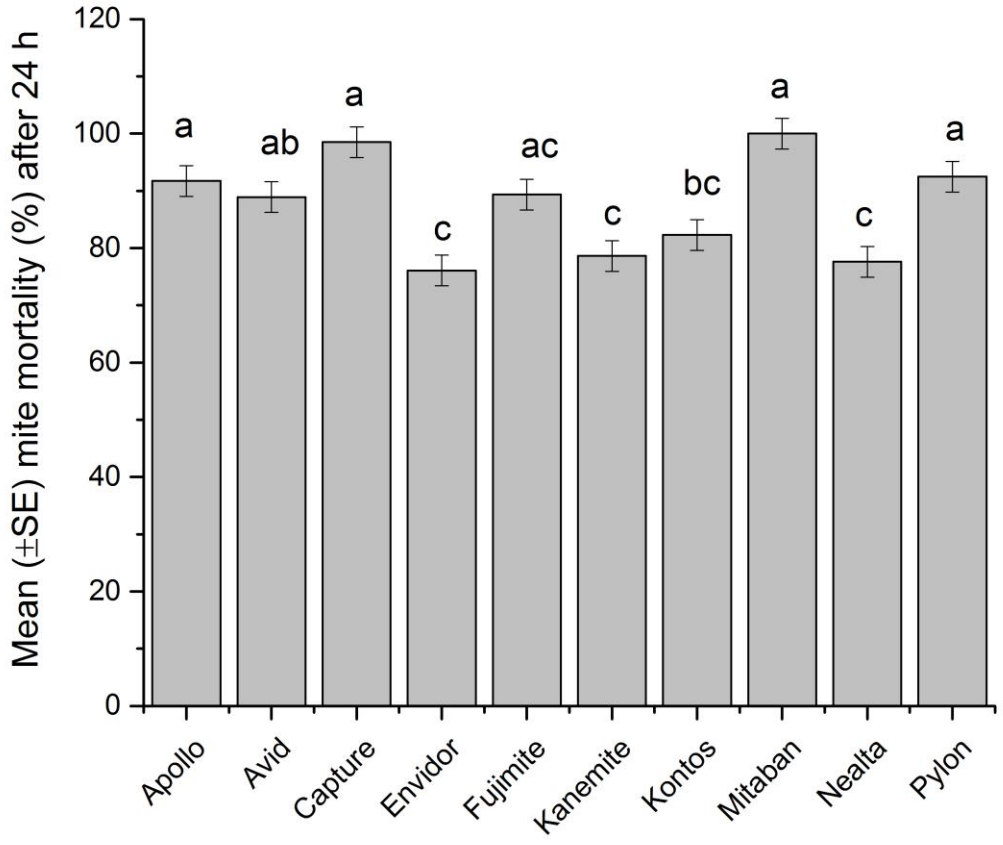

Fig. 27. Mean (±SE) daily mite drop exposed to tested formulated products after 24 h in glass vial assay (2017). Means with the same letter among treatments are not significantly different ($p<0.05$).

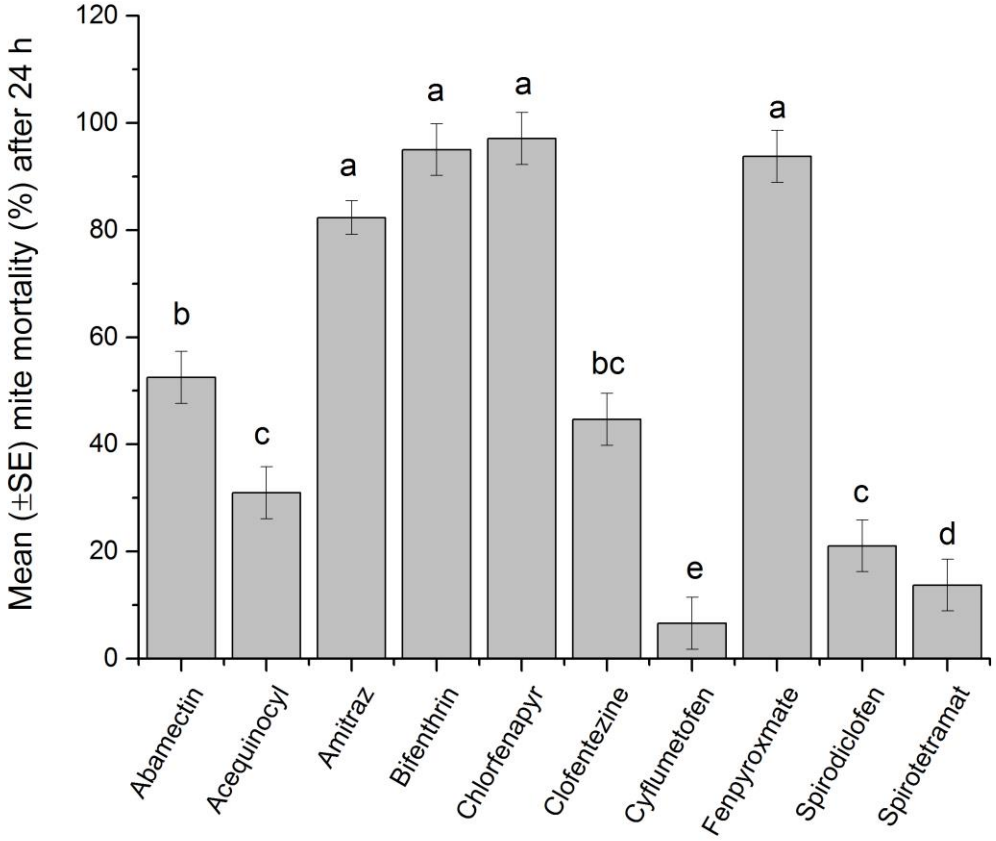

Fig. 28. Mean (±SE) daily mite drop exposed to tested active ingredients after 24 h in micro applicator assay (2017). Means with the same letter among treatments are not significantly different (p<0.05).

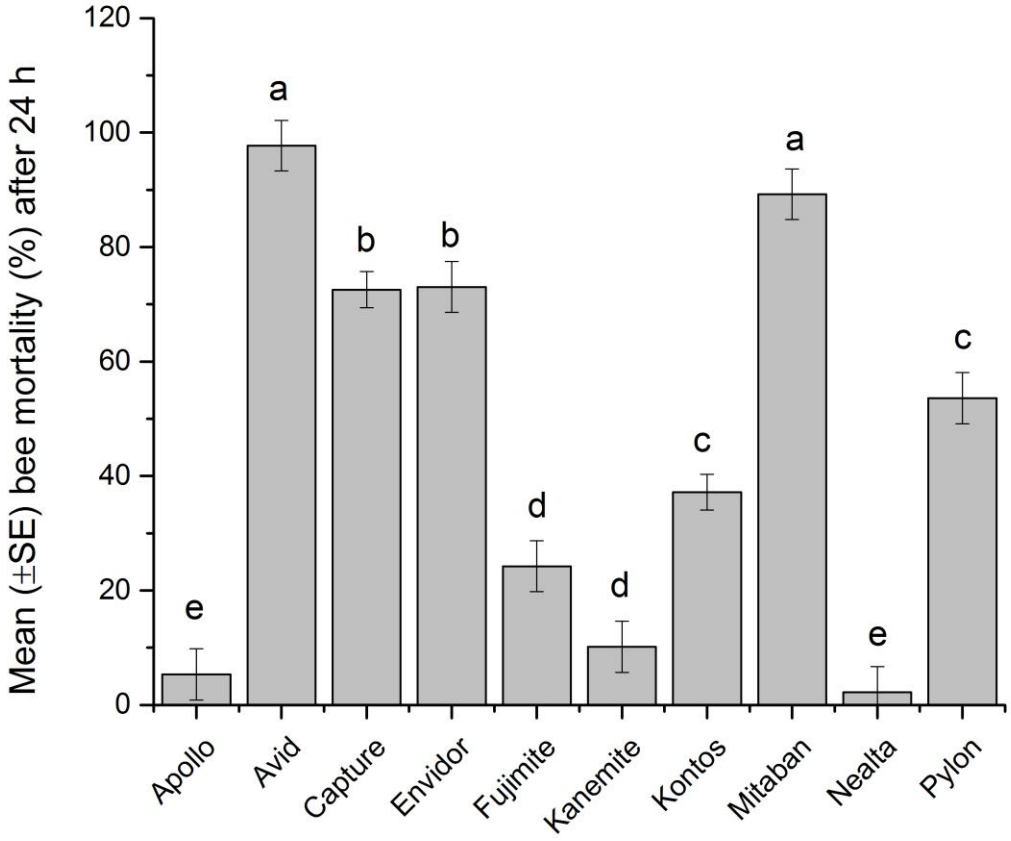

Fig. 29. Mean (±SE) daily bee drop exposed to tested formulated products after 24 h in Mason jar assay (2017). Means with the same letter among treatments are not significantly different (p<0.05).

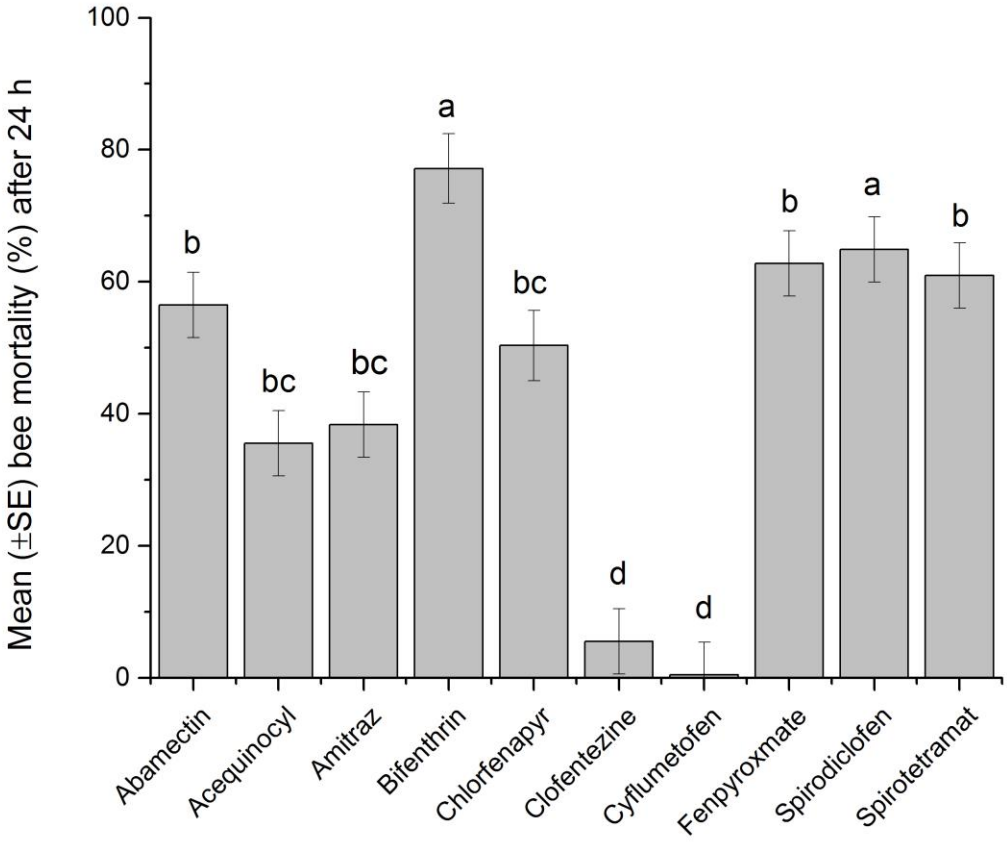

Fig. 30. Mean (±SE) daily bee drop exposed to tested active ingredients after 24 h in Mason jar assay (2017). Means with the same letter among treatments are not significantly different (p<0.05).

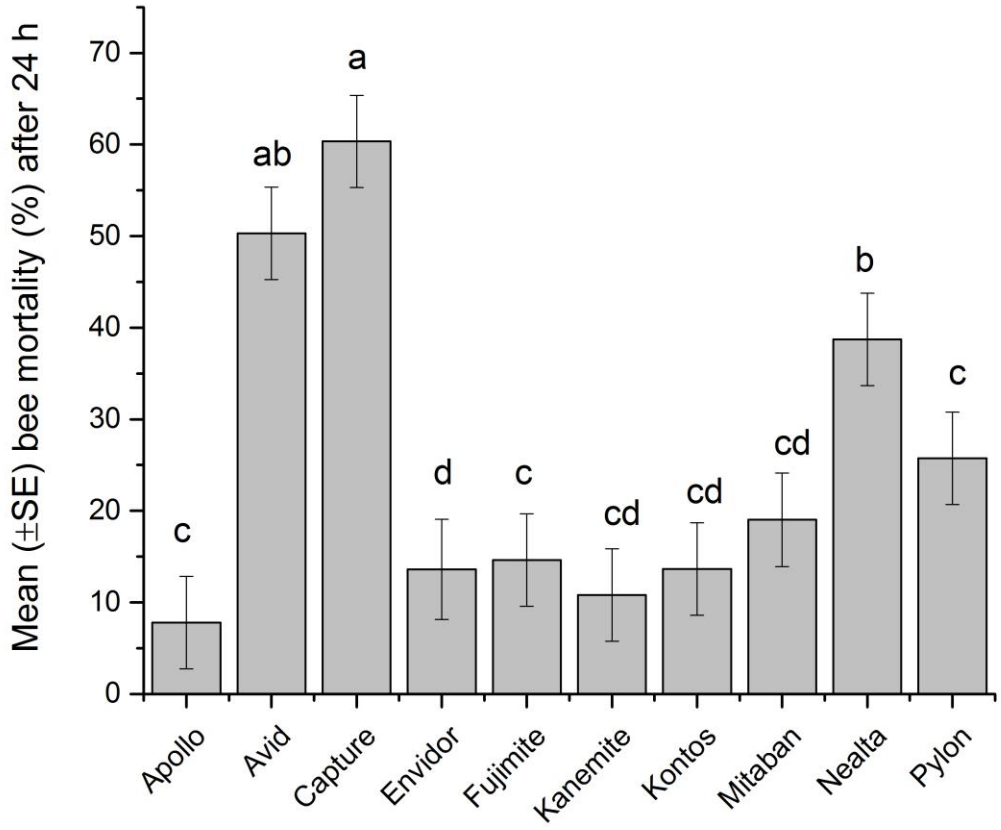

Fig. 31. Mean (±SE) daily bee drop exposed to tested formulated products after 24 h in micro applicator assay (2017). Means with the same letter among treatments are not significantly different (p<0.05).

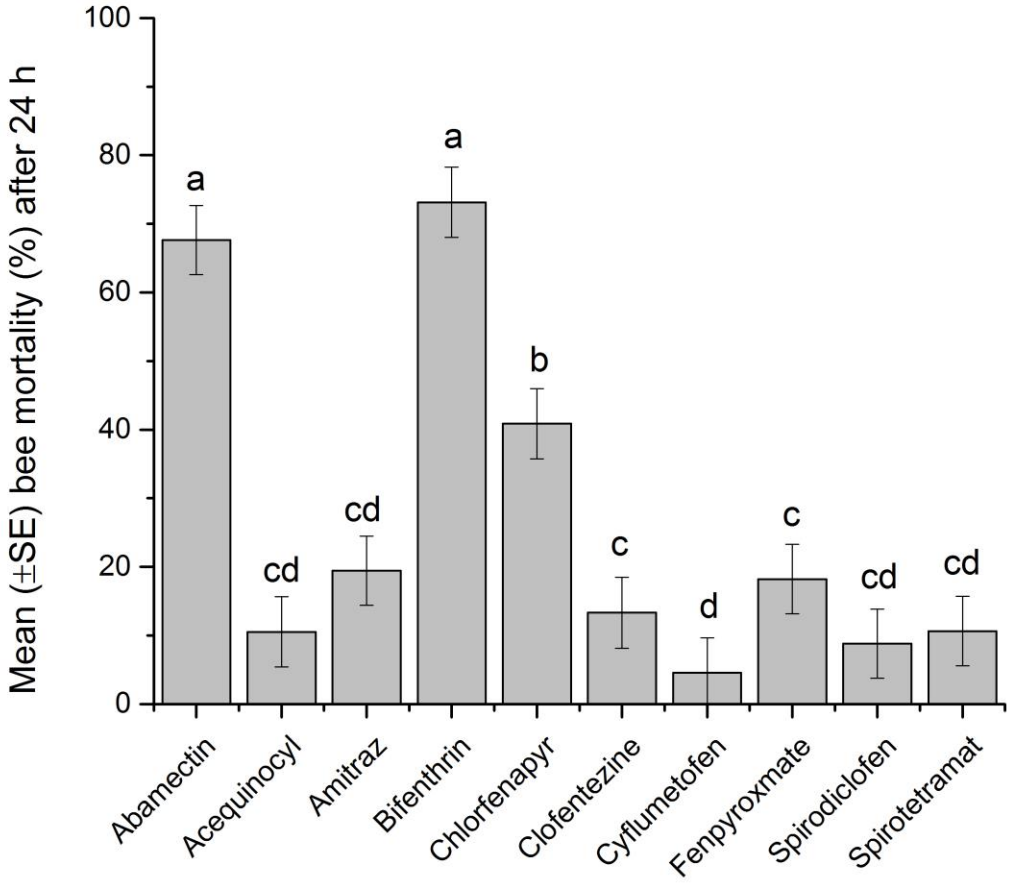

Fig. 32. Mean (±SE) daily bee drop exposed to tested active ingredients after 24 h in micro applicator assay (2017). Means with the same letter among treatments are not significantly different (p<0.05).

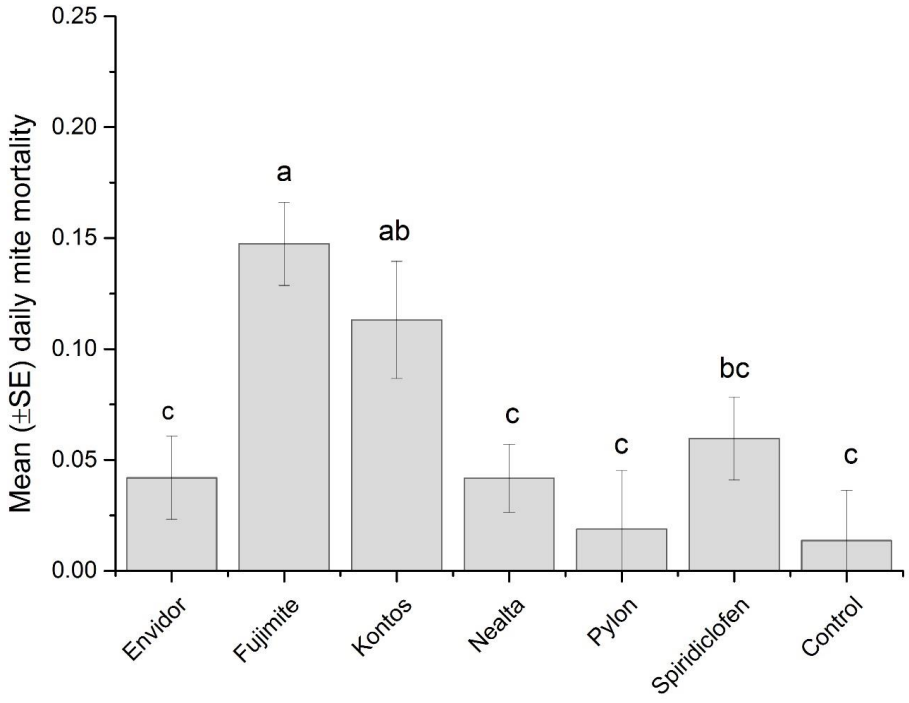

Fig. 33. Mean (±SE) daily mite drop exposed to tested formulated products and an active ingredient in the field (2017). Means with the same letter among treatments are not significantly different (p<0.05).

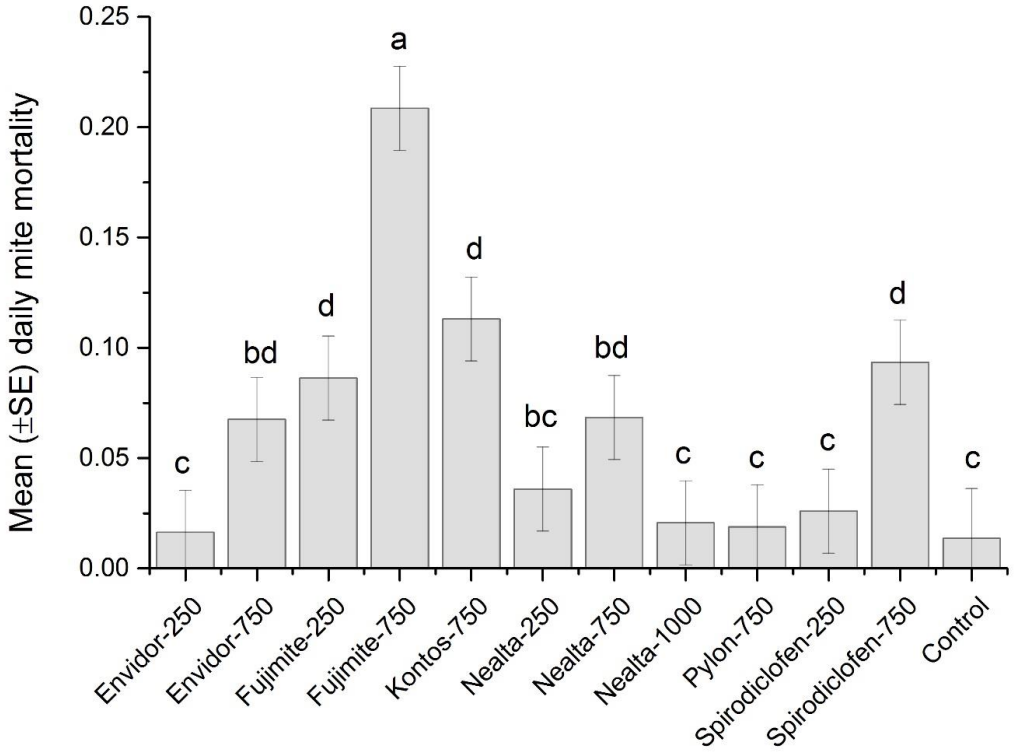

Fig. 34. Mean (±SE) daily mite drop exposed to different concentrations of tested formulated products and active ingredient in the field (2017). Means with the same letter among treatments are not significantly different (p<0.05).

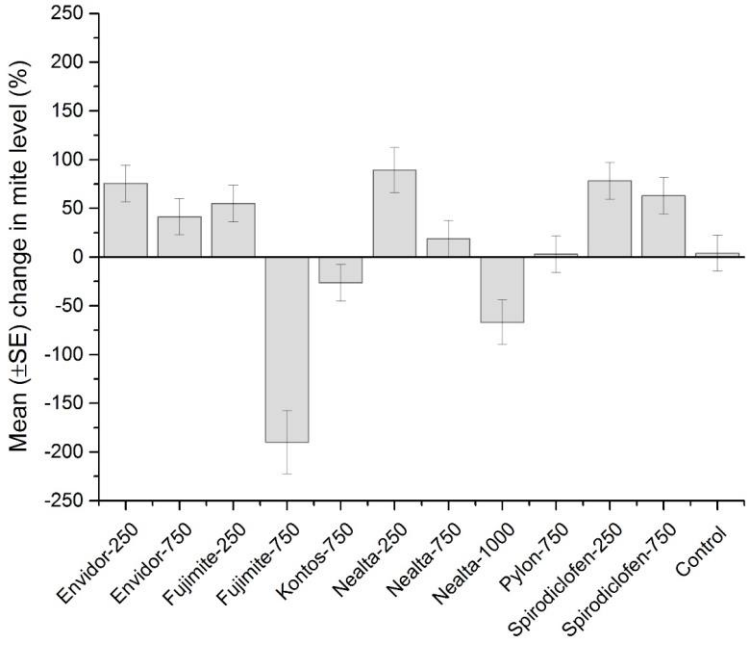

Fig. 35. Mean (±SE) changes in mite levels of treatments in the field study (2017).

Developing alternative new miticides to enhance the rotation system for sustainable control of Varroa mites.

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Alberta is the largest beekeeping industry in Canada, with over 40% of Canadian honey bee colonies (~315 000 colonies) found in the province. The value of honey sales in the last 5 years has ranged between $70-85 million per year (Stats Canada, 2017), and the honey bees' contribution to hybrid seed canola pollination as well as canola crop was estimated to be around $1.1 to 3.6 billion (Mark Darrach and Stephen Page, AAFC 2015).

Since 2007 Alberta beekeepers have experienced an average winter loss of 29% of their honey bee colonies (Canadian Association of Provincial Apiculturists (CAPA) 2007-2015). Between 2007 and 2009 however, bee colony winter losses reached a record high of 44% (CAPA 2008-2009). The major contributing factor to this high winter loss was the Varroa mite (*Varroa destructor*) because they developed resistance to the only two available synthetic miticides, Apistan® and Checkmite$^{+®}$. Although other options such as formic acid, oxalic acid, and Thymol have been available for beekeepers, their efficacy is variable and is dependent on a number of conditions: temperature, humidity, colony size, and whether or not there is brood in the colony (Imdorf et al., 1995).

To address Varroa resistance to applied miticides, the Alberta Apiculture program funded by Alberta Crop Industry Development Fund (ACIDF) investigated the efficacy of Apivar® and its potential side effects on bees. Apivar® at the time was another synthetic mite treatment developed for use in bee colonies. Results of the investigation showed that the efficacy of Apivar® exceeded 95%, with no side effects on bees, and non-harmful levels of chemical residues found in honey (Nasr el al., 2007). Collected data supported the

registration of Apivar® in Canada by the Pest Management Regulatory Agency (PMRA). The registration was intended for use by beekeepers to control Varroa mites already resistant to Apistan® and Checkmite+®. Since Apivar® was made available to Alberta beekeepers in fall 2008, beekeepers have become reliant on this single miticide for controlling Varroa levels in their colonies. So far, Apivar® has been successful in controlling mites and consequently, Albertan honey bee winter losses decreased by 50% in the past 4 years.

Once again beekeepers have come to rely on a single effective synthetic miticide (Apivar®) and the continued use of the same product year after year (since 2008) has raised serious concerns. In fact, the Alberta Apiculture program reported in 2016, the emergence of a small number of colonies with Varroa mites that have developed resistance to Apivar®. This discovery, though it was in a limited number of locations and only a slight emergence is again a cause for concern. Once Apivar®-resistant mites are able to reproduce and disperse to neighbouring colonies, these mites can spread and promote the propagation of Apivar®-resistant mites across the province.

These findings support the idea that the sustainability of bee health, the viability of the beekeeping industry, and supplying adequate number of bee colonies for crop pollination are uncertain. Therefore, the Canadian Honey Council, CAPA, Canada Standing Senate Committee on Agriculture and Forestry, and Canadian Bee Health Roundtable members have identified that the need for effective options for Varroa mite control are at the top of the priority list.

Development and evaluation of HopGuard as a potential varroa mite control agent under Alberta conditions.

Introduction

Hops (*Humulus lupulus* L.) derived compounds have been used as antimicrobial and disinfectant agents against various pathogens. These compounds contain beta acids that are known to be weak organic acids (Jones et al. 2003). They repel different plant pests that feed by sucking including the two-spotted spider mite (*Tetranychus urticae,* Koch) (Jones et al. 1996), and the hop aphid (*Phorodon humuli*, Schrank) (Hampton et. al. 2002; Jones et al. 2003). Hop beta acids also can reduce two-spotted spider mite oviposition and survival of adults (Jones et al. 1996).

Recently beta acids have been used in the United States to control Varroa mites in honey bee colonies (Degrandi-Hoffman et al. 2012). These extracts have shown to repel or kill phoretic Varroa mites with few or no adverse effects on honey bees during any of their life stages (Patent August 3, 2010). Phoretic means the stage of the mite lifecycle when it is feeding on adult bees outside of the brood cell. Degrandi-Hoffman et al. (2012) formulated a method to apply beta acids in bee colonies using cardboard strips (HopGuard™ - BetaTec Hop Products, Washington, DC, USA). When HopGuard™ strips were applied to bee packages installed in bee hives, more than 90% of the mites were killed without an increase in bee mortality (Degrandi-Hoffman et al. 2012). However, the presence of brood in a bee colony substantially decreased when HopGuard™ was applied. Therefore, HopGuard™ was best used in periods where there was minimal brood in colonies; the fall or in installed bee packages during early spring.

Further studies showed that HopGuard™ has limited efficacy under Albertan conditions when applied to reproducing colonies (Vandervalk et al. 2014). As brood was present, the efficacy for HopGuard™ in the fall and spring trials were just under 43% and just over 43% respectively. These reported efficacy levels were much lower than the efficacy of 93.5% reported for HopGuard™ in Germany (Rademacher and Harz

2011). Vandervalk et al. (2014) explained the low efficacy reported in her Albertan study could be caused by the presence of brood and a limited period of bee exposure to HopGuard™ strips. Honey bees chewed and removed much of the applied HopGuard™ cardboard strip with few days of treatment.

Despite HopGuard™ not causing enough mite mortality to be an effective treatment for full size colonies with brood, its present formulation has shown potential in niche areas such as the treatment of packages of bees (DeGrandi-Hoffman et al. 2012), or broodless colonies (Rademacher and Harz 2011). Therefore, the objectives of the Alberta Apiculture program experiments were as follows:

1. improve the delivery system to enhance the efficacy of HopGuard™
2. determine efficacy of HopGuard™ in modified application methods "HopGuard II™" on Varroa mites and any side effects on honey bees
3. determine beta acid residues in honey after application of HopGuard II™ to honey bee colonies under Alberta conditions.
4. assemble all results and prepare a registration package to PMRA for use as a miticide in Canadian bee colonies.

Experiment 1. Evaluation of HopGuard™ efficacy and determination of the mode of action against Varroa mites, summer.

Objectives of this experiment was conducted to:

1. Determine the effect of repeated application of HopGuard™ on the efficacy against Varroa mites in comparison to a single application.
2. Determine the mode of action of HopGuard™ against Varroa mites and whether HopGuard™ material needs to come into contact with the bees to kill mites.
3. Evaluate which conditions need to be met for HopGuard™ strips to be effective under Alberta's conditions.

Materials and Methods

This experiment was conducted at the Crop Diversification Centre North (CDCN), Edmonton, Alberta, Canada, Forty honey bee colonies infested with Varroa mites were used in this experiment. Colonies were housed in double chambered Langstroth hives situated on Apinovar screened bottom boards with a removable tray (Chapleau 2003). Overwintered colonies headed with Kona Hawaiian queens were used. Sugar syrup (1 sugar: 1 water) in frame 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. Colonies were free from *Paenibacillus larvae* (American foulbrood) symptoms during the trial, and samples were taken periodically to determine *Nosema* spp. infection. The experiment ran from July 30 – October 8.

Determination of Varroa infestation levels.

The alcohol wash method was used to assess Varroa mite infestation (De Jong et al. 1982). Samples of approximately 200-300 Honey bees were removed from brood combs and preserved in 70% ethanol. The samples were then agitated on a 175 rpm orbital shaker for 15 minutes and rinsed repeatedly with running water using a 12 mesh strainer. Individual Varroa mites fell through the strainer and were subsequently collected in an 11 Litre Rubbermaid® basin and counted. The honey bees in the strainer were weighed to determine the number of Honey bees in the sample. This was calculated based on the average weight of a single bee as determined by weighing three samples of 10 honey bees per sampling date. The number of Varroa found in the sample was then divided by the calculated number of honey bees to determine the infestation percentage.

Varroa mite mortality for each colony was monitored using sticky traps (Mann Lake, Hackensack, MN, USA) placed on the tray beneath the Apinovar screened bottom board (Chapleau 2003). Dead or dying Varroa in the colony fell through the screen onto the sticky traps. Sticky traps were removed and

replaced every first, second, third, and fifth day after treatment. The collected sticky traps, covered in plastic wrap, were returned to the lab and adult Varroa were counted. Only Varroa within the printed grid on the sticky board were counted. Varroa mortality per day was calculated by dividing the number of Varroa found on each sticky trap by the number of days the sticky trap was in place.

Colony strength assessments

Colony strength assessments were performed to estimate the area of capped brood, the area of frames covered with adult bees, and the amount of stored honey for each colony. The area of one side of a standard Langstroth comb was measured to be 900 cm2; therefore the area of brood, bees, and honey was recorded by visually estimating what proportion of the entire comb was covered by each to the nearest 30 cm2 (Skinner et al. 2001). All combs in the colony were removed and both sides were estimated visually.

Treatments

A complete randomized block experimental design was used when assigning treatments to account for variations in colony strength, brood area and initial mite infestation. A block of 5 bee colonies was assigned randomly to a tested treatment. Eight treatments were tested as follows:

1. 15g of liquid HopGuard™. 15 g of liquid HopGuard were placed in a Petri dish and replaced every 10 days, for a total of 2 applications
2. 15 g of liquid HopGuard™ mixed with 2.5grams thymol powder. 15 g of liquid HopGuard™ and 2.5 g of thymol powder were placed in a Petri dish. HopGuard and thymol powder were replaced every 10 days for a total of two applications. Thymol powder was mixed in to make HopGuard™ more palatable to bees.
3. 2.5 grams of thymol powder. 2.5 grams of thymol powder were placed in a Petri dish. Powder was replaced every 10 days for a total of two applications

4. Screened HopGuard™ strips. 4 HopGuard™ strips were placed in two screened off frames to prevent bees' access to HopGuard™ material. Strips were replaced every 5 days for a total of 4 applications (Fig. 1)
5. 3 days incubated at 25C HopGuard™ strips. HopGuard™ strips incubated at 25C for 3 days prior to application were tested to find out if HopGuard™ continues to be effective after this incubation period (Fig. 2). Two strips were placed in the bottom brood chamber and 2 were placed in the top brood chamber. Strips were replaced every 5 days for a total of 4 applications
6. 5 days incubated at 25°C HopGuard™. HopGuard™ strips were exposed in an incubator set at 25°C for 5 days prior to placement in the colony to find out if the Hops extract was still effective. Two strips were placed in the bottom brood chamber and 2 were placed in the top brood chamber. Strips were replaced every 5 days resulting in 4 applications in total.
7. HopGuard™ strips: 2 strips were placed in the bottom brood chamber and 2 strips were placed in the top brood chamber. The strips were replaced every 5 days for a total of 4 applications (Fig.3).
8. No treatment

Finishing treatment

Apivar® was used as a finishing treatment to quantify the number of Varroa remaining in the colonies to calculate efficacy of the treatment. 20 days after treatments were applied, HopGuard™ strips were removed and replaced with Apivar® (one strip for every four to five frames covered with bees). Apivar strips were left in the colonies for six weeks as directed on the packaging. Sticky traps were inserted on the first, second, third and fifth days after the initial treatment and every five to seven days following to determine Varroa mortality during the finishing treatment period.

Fig. 1. Screened HopGuard™ strips placed in bee colonies to prevent bees from contacting HopGuard™ materials.

Fig. 2. Incubated strips at 25°C to dry the strips before applying to bee colonies.

Fig. 3. HopGuard™ strips applied to bee colonies (1 strip per 5 frames covered with bees).

Efficacy of each treatment was calculated according to the following formula.

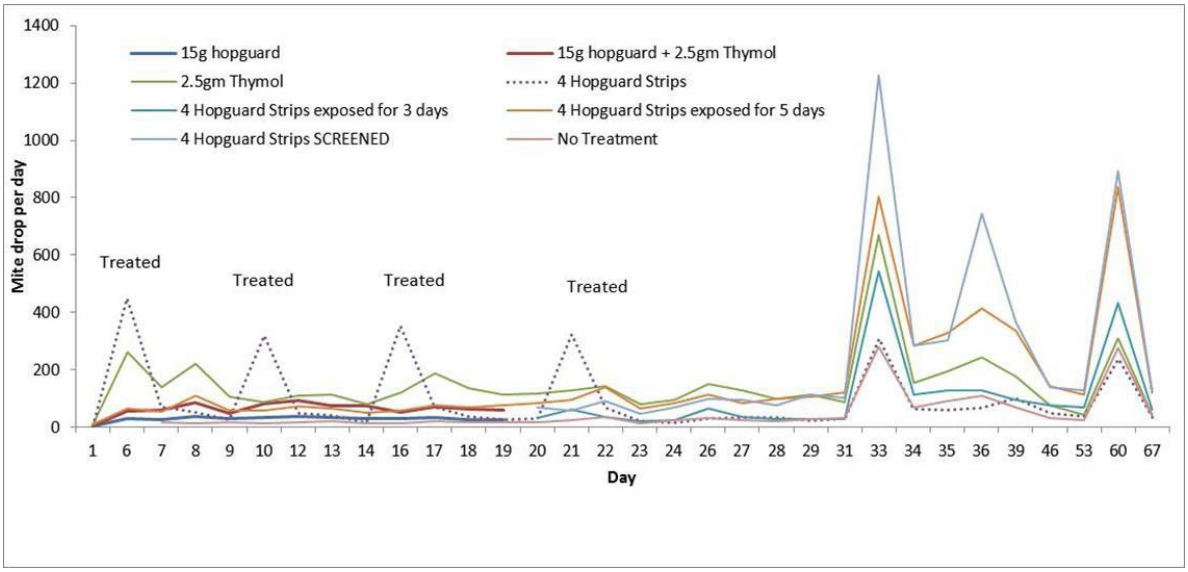
$$\text{Efficacy} = \frac{\text{mites killed by treatment}}{\text{mites killed by treatment} + \text{mites killed by finishing treatment}} \times 100$$

All analyses were completed using SAS 9.3 for Windows (SAS Institute 2011). The effect of treatment on Varroa population assessments was analyzed with repeated measures analysis of variance with the treatment model as a fixed effect and the pre-treatment readings used as a covariate (PROC MIXED, SAS Institute 2011). Efficacy was calculated according to the above 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. A comparison of frames covered with bees and calculated brood areas was conducted between pre-treatment and after treatment to determine any side effects of applied treatments on honey bees.

Results

Varroa drop averaged 17 mites per day prior to treatment. After the first application of tested treatments, the daily mite mortality increased to up to 686 per day. High levels of mite mortality were observed during the first three days after application of treatments of HopGuard™ strips (Fig. 2). Each time a new application of HopGuard™ was applied, high mite morality was reported the first three days after treatment. The efficacy of treatment was significant among treatments (P=0.0421, Table 1). Multiple applications of HopGuard™ had the highest efficacy (56.7± 16.9%), and the treatment of 2.5 g of thymol powder being the next highest (42.2± 14.6%). The 3 and 5 day incubated HopGuard™ strips at 25°C had insignificant efficacy on mite mortality (11.3±5.1% and 20.5±4.2%). Screened HopGuard™ strips did not affect mite mortality when compared with control treatment colonies (9.4±2.2%). The amount of frames covered with bees and brood area did not show significant differences between treatments.

Fig. 4.



Daily mite drop in first trail using various tested treatments in Alberta.

Table 1**.** Estimated Efficacy of tested treatments in comparison to untreated control.

Treatments	# Treatments	Average±STD Efficacy (%)
4 Hopguard Strips	4	56.7±16.9*
4 Hopguard Strips incubated for 3 days	2	11.3±5.1
4 Hopguard Strips incubatd for 5 days	4	20.5±4.2
4 Hopguard Strips SCREENED	2	9.9±2.0
15g hopguard liquid	2	10.8±3.7
15g hopguard liquid+ 2.5gm Thymol	2	9.4±2.2
2.5gm Thymol	4	42.2±14.6*
No Treatment	N/A	10.7±4.0

Discussion

Honey bees that were not able to contact the cardboard strips to get HopGuard™ material on their bodies, did not show any difference in mite mortality when compared to untreated colonies. This result confirms that HopGuard™ is a contact miticide. When applied to contact bees and mites, HopGuard™ significantly increased the daily mite mortality in treated colonies. However, the cardboard HopGuard™ strips dried quickly and lost their efficacy in killing mites. Once strips dried, honey bee tended to chew and discard them from the colony. Multiple applications of HopGuard™ strips are needed to get the highest efficacy. Our results showed that HopGuard™ applied once every 5 days at least 4 times showed the highest efficacy against Varroa without impacting bee brood production and adult bee populations.

Based on these research results and previously reported results by Vandervalk 2013, HopGuard™ has a short period of time where it can be an effective contact-miticide. Repeated applications of HopGuard™ are required to increase the efficacy for mite control. This increased number of applications is considered expensive and time consuming by beekeepers. Thus, there is a need to improve the application method and change the substrate of applied strips to increase exposure time of the active ingredient on bees to more than 3 days. A stronger substrate would remove the risk of bees chewing the treatment. These improvements would reduce number of time consuming applications and be more cost effective for beekeepers.

Experiment 2. Development and evaluation of new HopGuard II™ strips to improve efficacy against Varroa mites, fall 2013.

Based on Experiment 1 results, most of the mites during the evaluation test were killed during the first 2 days of application of HopGuard™ strips to bee colonies. After the first two days, the strips quickly dried out, and in some cases the bees chewed and removed them from the hives. The reported high kill of Varroa mites within the first two days of application encouraged our team to follow up and attempt to improve HopGuard's™ application method.

The main objectives of this experiment were:

1. To develop a new strip that is hard for bees to chew and can deliver a large amount of Hop Beta acids to bees in bee colonies to improve efficacy against Varroa mites.
2. To assess the possible side effects on honey bee populations and brood.

Materials and Methods

Developing a new H*opGuard™ strip to apply in bee colonies.*

Beta Tech-Hop products company worked with specifications provided by the Alberta research team to improve HopGuard™ applications. Several substrates were selected and preliminarily tested in bee colonies. The criteria to be used in selection of the new substrate were: 1) a substrate can carry up to 25 g of HopGuard™ material, and 2) substrate is stiff, hard and cannot chewed by honey bees.

Field experiments to test the newly developed HopGuard II™.

Field testing of the new HopGuard II™ was conducted in 55 honey bee colonies in Alberta and Saskatchewan. To test the efficacy under different management systems, thirty double brood chamber colonies were used in Alberta, and 25 single brood chamber colonies were used in Saskatchewan. All colonies were managed using standard beekeeping management methods. An experimental design similar to the first experiment was used to determine the efficacy of HopGuard II™. A complete randomized block

experimental design was used, which accounted for colony strength, brood area, and initial mite infestation. A block of bee colonies was randomly assigned to a treatment group.

Treatments

1. Old - 4 original old HopGuard™ strips (1 strip for every 5 frames covered with bees). Strips were replaced every 5 days, 3 times
2. New 1 - 4 newly designed HopGuard II™ strips applied once to colonies (1 strip for every 5 frames covered with bees).
3. New 2 - 4 newly designed HopGuard II™ strips (1 strip for every 5 frames covered with bees). Strips were applied twice; once every 10 days
4. New 3 - 4 newly designed HopGuard II™ strips (1 strip for every 5 frames covered with bees). Strips were applied 3 times; once every 6 days
5. Control - Non treated control

All tested treatments were the same in Alberta and Saskatchewan, except treatment 4 and 5 were not included in Saskatchewan experiment. Thirty days after application of HopGuard™, a finishing treatment was applied to calculate the relative efficacy of each tested treatment. Apivar® was used as a finishing treatment for 42 days in Alberta, and for 12 days in Saskatchewan. Sticky traps were used to determine the daily Varroa mite mortality during the testing period of applied treatments, and the finishing treatment period of Apivar®. When an application was applied, the sticky traps were replaced at day 1, 2, 3, 5, and every 5 days thereafter. All mites that fell on sticky traps were counted to determine the daily mite drop, and efficacy of each tested treatment. The bee population and brood areas were measured every two weeks by assessing areas covered with bees, brood and honey. Samples of approximately 200-300 bees were collected from brood combs of each experimental colony and Varroa mites were determined in each sample of bees as described by DeJong (1982).

The efficacy of tested treatments were calculated according to the following formula:

$$\text{Efficacy} = \frac{\text{mites killed by treatment}}{\text{mites killed by treatment+mites killed by finishing treatment}} \times 100$$

All analyses were completed using appropriate statistical methods as performed by SAS 9.3 for Windows (SAS Institute 2011). A comparison of frames covered with bees and calculated brood areas was conducted between pre-treatment and after treatment to determine any side effects of applied treatments on honey bees. Trials ran from September 30 to November 24, 2013.

BetaTec Hop Products preliminarily screened several materials to use in HopGuard II™. After consultation with the Alberta Bee Team, one substrate was selected (Fig. 2). Potassium salts of Hops beta acid was applied to the strips (25 g/strip) and the newly developed modified strips (HopGuard II™) were delivered to Alberta for field testing.

Fig 1. New substrate of HopGuard II™.

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Fig. 2. Applying HopGuard II ™ strips to bee colonies

Results

The daily average natural Varroa mite mortality was 24.6 per day prior to treatment. After the first application of the newly developed HopGuard II™ treatments, the mite mortality during the first day averaged 1806 in treated colonies, and 76 mites in control colonies. The daily rate of mite mortality in tested treatments are presented in Fig. 3. The newly developed HopGuard II™ resulted in high mite mortality for 3-5 days following each application. The average daily mite drop was significantly different among

treatments (F=82.27, df= 4, 30, p<0.0001) (Fig. 4.). Multiple applications of HopGuard II™ had significantly higher daily mite mortality rates than multiple applications of HopGuard™, or a single application of HopGuard II™ (p<0.05).

The efficacy of treatments among HopGuard II™ and HopGuard™ was significantly higher than controls (F 151.78, df=4. 30, p<0.0001). The efficacy was 92.0%±5%, 97.0% ± 13.3%, and 92.3% ±4.1% for HopGuard II™ with 2 applications and HopGuard II™ with 3 applications, and HopGuard™ with 3 applications (once every 5 days), respectively.

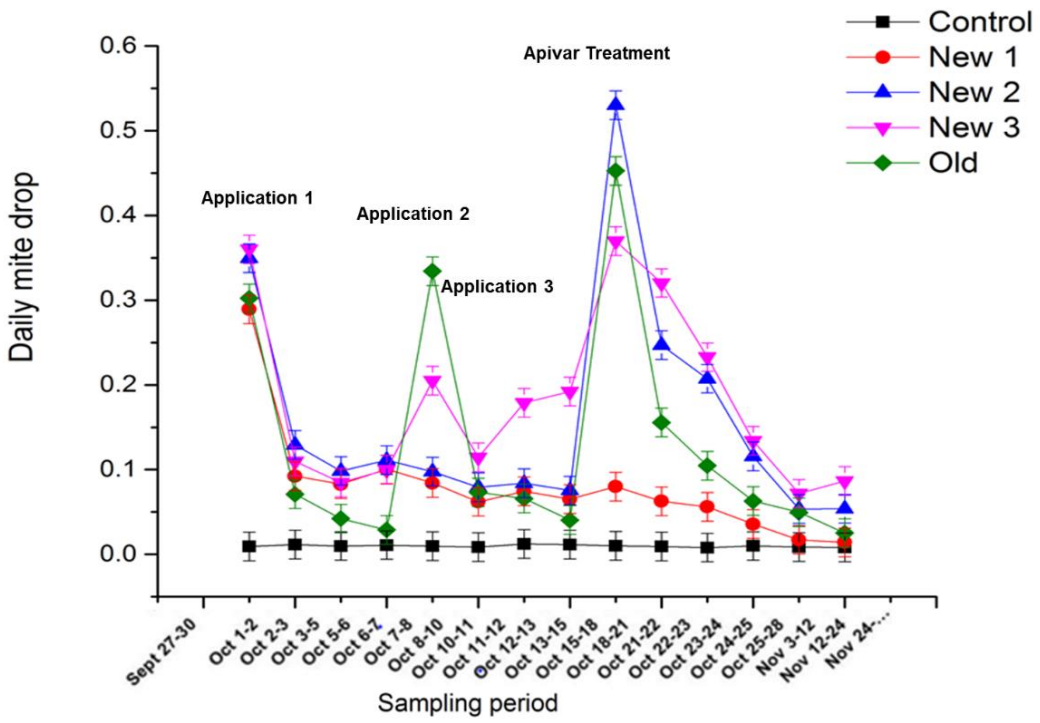

Fig. 3. Daily mite drop in first trial in Alberta.

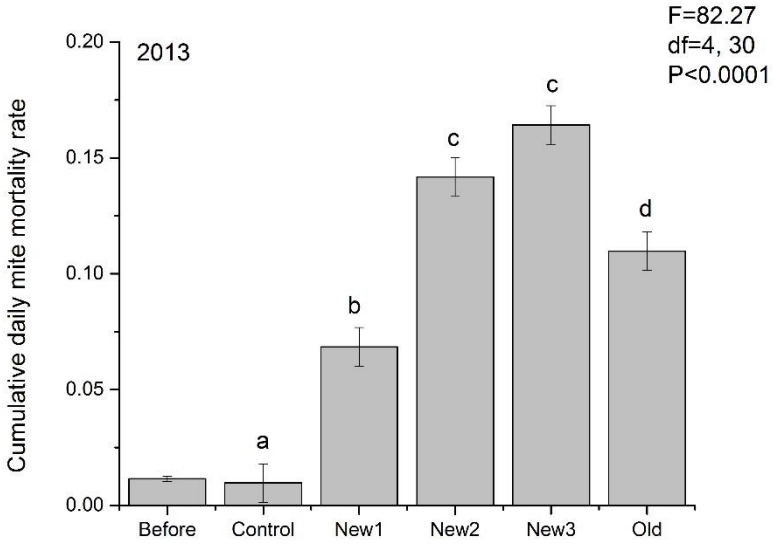

Fig. 4. Cumulative daily mite drop in Alberta.

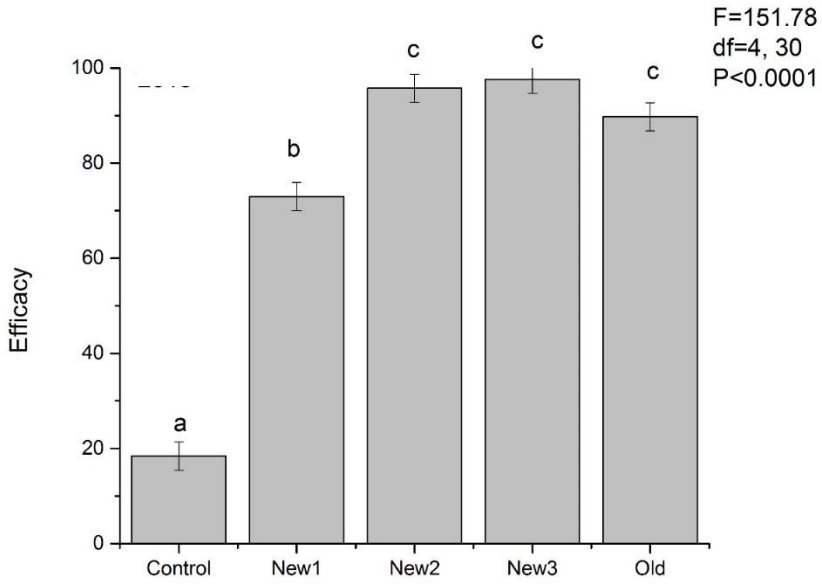

Fig. 5. Efficacy of newly developed HopGuard II™ in Alberta.

Discussion

When compared to HopGuard™, the newly developed HopGuard II™ showed a significant improvement in efficacy of Varroa control. The replacement of the substrate increased of the time of activity to up to 5 days, which increases the exposure of bees to the active ingredient of HopGuard II™. There is also an increase of active ingredients applied to each colony. This could contribute to the longer lasting activity of HopGuard II™ compared to HopGuard™, which allows more emerging mites to be killed for longer periods. A single treatment of HopGuard II™ caused a peak in *V. destructor* mortality the day of treatment, which was followed by a subsequent lower daily mite mortality that lasted for 30 days. The finishing treatment with Apivar showed insignificant mite drop after a single application of HopGuard II™. Although a single application of HopGuard II™ lasted long enough to kill emerging mites, multiple applications of HopGuard II™ increased the efficacy up to 96%. Increased efficacy of mite control means HopGuard II™ offers better protection to honey bees than HopGuard™.

This study determined the efficacy of HopGuard II™ against Varroa mites. The new substrate and increased dose (25 g per strip) have resulted in higher efficacy that could better protect honey bees from Varroa mites. HopGuard II™ can be an effective tool for Varroa control in an integrated pest management system for beekeepers. Because this newly developed miticide has a different mode of action to currently available miticides, it can be an effective alternative for mite control and can be used for management of resistance to miticides. The availability of HopGuard II™ for Varroa control will help beekeepers maintain healthy colonies for crop pollination and honey production.

All colonies were tested for *Nosema apis* and *Nosema ceranae* disease levels according to Reuter et al. To test for Nosema levels, samples of approximately 100 bees were taken from each hive and stored in 70 % alcohol. The abdomens of 30 workers from each sample were ground in a tissue grinder with 15 mL of water, and then examined under a light microscope with a haemocytometer. The number of Nosema

spores found in the four corner squares and the centre square of the Neubauer grid on the haemocytometer was multiplied by 25,000 in order to obtain the spore load per bee.

Experiment 3. HopGuard II™ under-over dosage and application, summer 2014.

Objective

The purpose of this experiment was to build on the previous 2 experiments and investigate the effects of overdosing and under dosing Honey bee colonies with HopGuard II™ strips. In addition, the experiment further investigated the impact HopGuard™ in its new formulation had on Varroa mite populations, honey bee populations and summer brood production.

Methods and Materials Eight treatment groups with six reps per group, for a total of 48 colonies, were set up for the experimental start date of June 16, 2014:

1. ½ a strip of HopGuard II™ for every 5 frames of bees, applied once.
2. ½ a strip of HopGuard II™ for every 5 frames of bees, applied twice; 10 days apart.
3. 1 strip of HopGuard II™ for every 5 frames of bees, applied once.
4. 1 strip of HopGuard II™ for every 5 frames of bees, applied twice; 10 days apart.
5. 1.5 strips of HopGuard II™ for every 5 frames of bees, applied once.
6. 1.5 strips of HopGuard II™ I for every 5 frames of bees, applied twice; 10 days apart.
7. 2 strips of HopGuard II™ for every 5 frames of bees, applied once.
8. Control; No treatment.

HopGuard II™ strips were placed in the bottom and top brood chambers of experimental colonies. As a finishing treatment, Apivar® strips were put in all colonies according to the labelled instructions. Treatment groups of only one HopGuard II™ application or no treatment received their finishing treatment on June 26, 2014. All colonies receiving a second application of HopGuard II™ received the Apivar® treatment on July 6, 2014.

Colonies were scored for strength (bees, and brood) before the experiment start date (June 11 and 12), prior to any additional treatments to colonies (June 24-25, July 3-4), and following all treatments when the final Apivar strips were removed from colonies (August 18-19). Scoring involved removal and examination of each frame in the colony, where the square inches of brood and the percentage of the frame covered in bees was estimated.

In addition to colony strength, Varroa infestation (%) for all experimental colonies was determined: prior to treatment, after first treatment, after second treatment, and after the finishing Apivar treatment (Table 1). This was done by taking a sample of approximately 300 bees from a brood frame and washing the sample to determine number of mites and number of bees. The number of bees was estimated by the average weight of 3 samples of 10 bees (see previous experiments). Mite drop was determined using Varroa Mite sticky traps (Contech Enterprises Inc., Canada). They were placed in a Propolis-brand screened bottom boards. Sticky boards were replaced and visually counted every 1,3,5,7 days depending on treatment times.

Table 1. Treatment groups and application dates for experimental colonies.

Date and Treatment applications for Summer 2014	**Graph Legend**	**June 16 First Treatment**	**June 26 Second Treatment**	**June 26 Apivar**	**July 6 Apivar**
Half strip of HopGuard II™ applied once	half1	X	–	X	–
Half strip of HopGuard II™ applied twice	half2	X	X	–	X
One strip of HopGuard II™ applied once	One1	X	–	X	–
One strip of HopGuard II™ applied twice	One2	X	X	–	X
One and a half strips of HopGuard II™ applied once	Onehalf1	X	–	X	–
One and a half strips of HopGuard II™ applied twice	Onehalf2	X	X	–	X
Two strips of HopGuard II™ applied once	Two1	X	–	X	–
No HopGuard Treatment	Control	–	–	X	–

(X) Received Treatment, (-) No Treatment administered

Results

In all cases the efficacy of two applications of HopGuard II™ was higher than one application; Half the recommended dose of a HopGuard II™ applied once versus twice was 33% and 72% respectively, the recommended dose of HopGuard II™ (1 strip per 5 frames of bees) applied once versus twice was 34% and 79% respectively, and one and a half times the recommended dose applied once versus twice was 36% and 65% respectively (Fig. 1). There was no significant difference between the efficacies of halving the dose and applying the recommended dose once (Half 1; 33%, One 34%, Fig.1). However when the dosage was double of what is recommended (2 strips per 5 frames of bees), the efficacy was 46%.

The number of applications of HopGuard II™ and the dose of the application did not show significant differences in adult bee populations and brood production except in one treatment (Fig. 2 and 3). The treatment where HopGuard II™ was two times the recommended dose showed a large decrease in adult bee population (Fig. 3).Brood present in the colony decreased for all treatments but were not noticeable different from the decrease also present in the non-treatment control group.

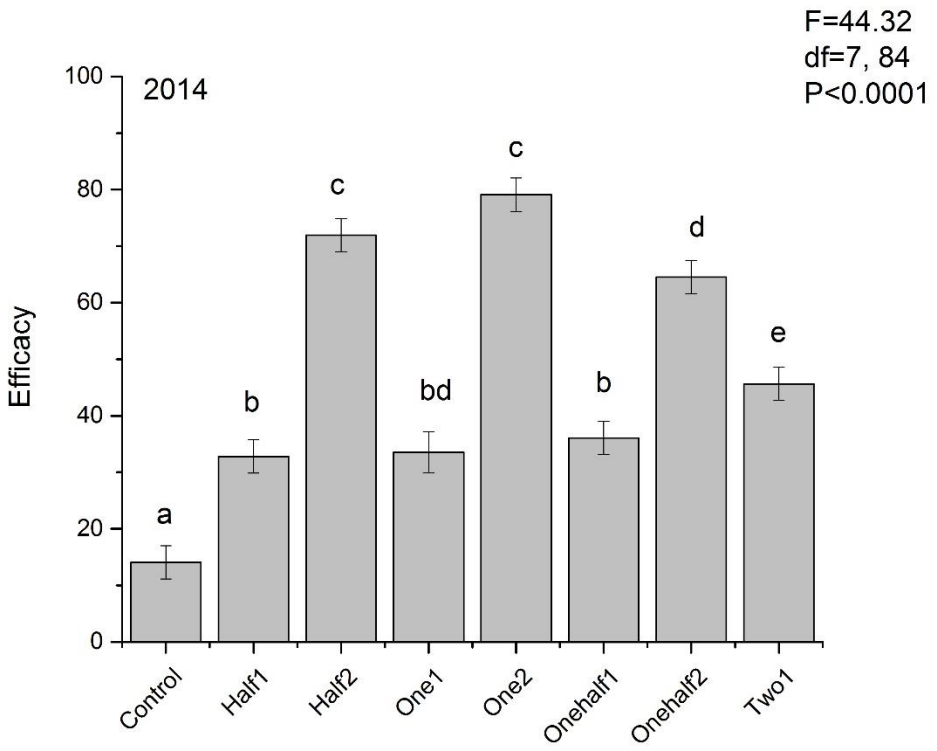

Fig. 1. Efficacy of treatments.

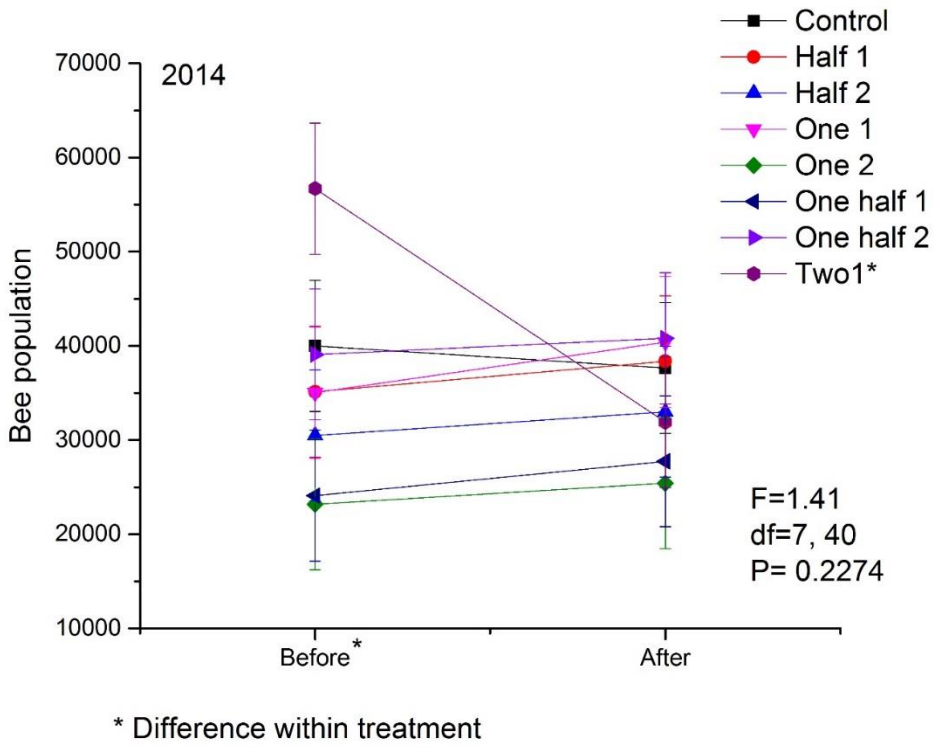

Fig. 2. Honey bee population before and after treatments.

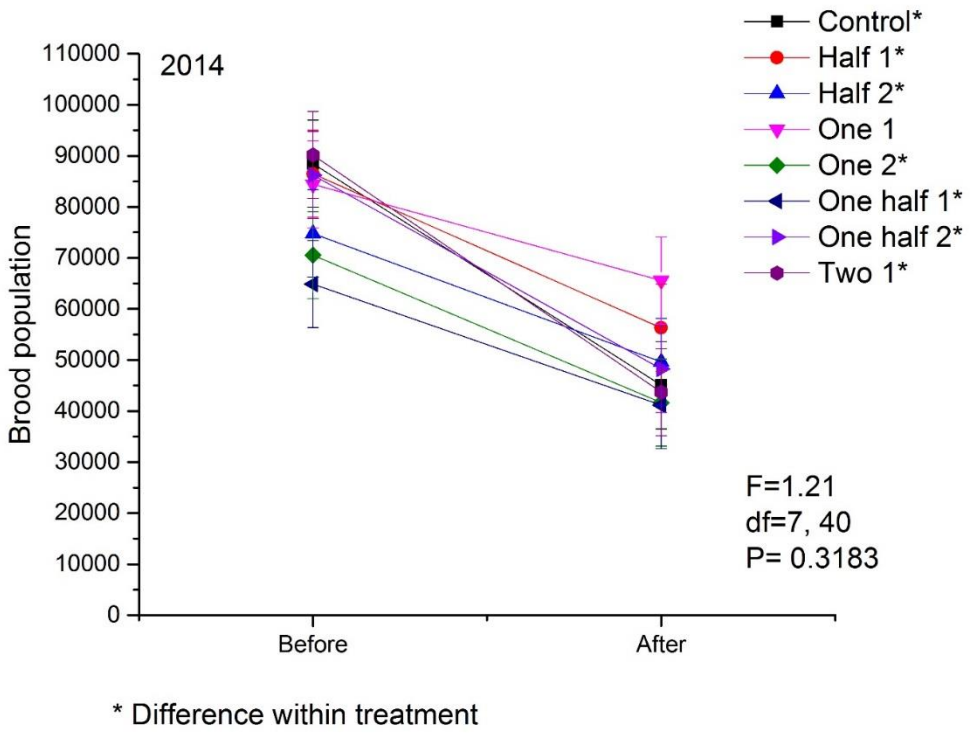

Fig. 3. Brood population before and after treatment.

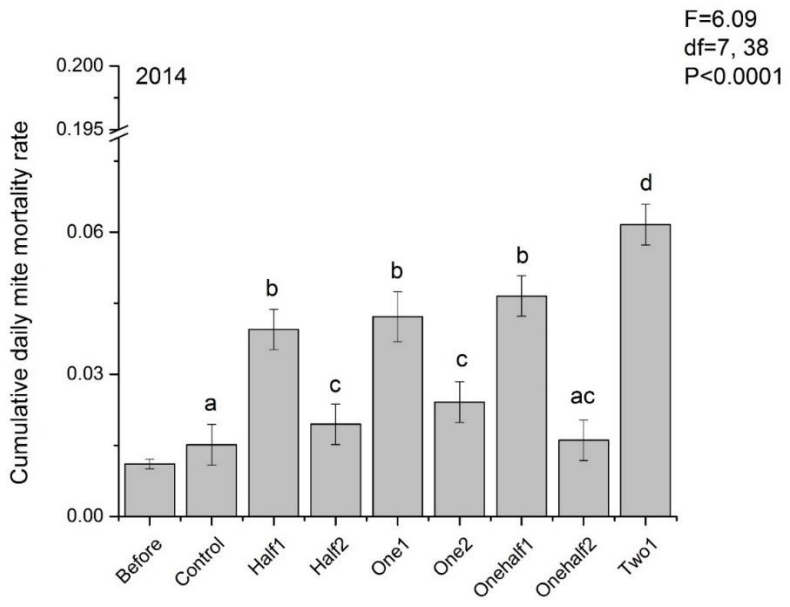

Fig. 4. Cumulative daily mite mortality for HopGuard II™ treatments.

Discussion

Our experiment showed that two applications of HopGuard II™ were significantly more effective at reducing mite populations than a single application. When dosage was added, the most effective dose for killing mites was double the recommended dose applied once, however there were effects on adult honey bee population. One application of the recommended dose or half of the recommended dose showed minimal mite kill but two applications of these doses showed the highest efficacy of treatment with no significant effects on adult bee population and brood production. Therefore, the recommended dose at two applications would be the most effective at removing mites while still maintaining regular brood and adult bee production.

Experiment 4. HopGuard II™ concentration, application and honey residues, summer 2015.

Objectives

As previous experiments have shown efficacy in months where brood production is lower (spring and fall), this experiment aimed to test HopGuard II™ strips in colonies at the peak of brood and honey production. The aim of the experiment was to test HopGuard II™ strips at different concentrations, in single or double applications to determine the efficacy of varroa mite control in honey bee colonies with brood present. How the different concentrations and number of applications impacted the production of brood, bees and honey was also investigated. In addition, the manufacture of HopGuard II™ claims that it is safe to use during the honey flow because it doesn't leave residues in extracted honey (Ahumada-Segura, 2010b). Extracted and brood chamber honey were both tested to establish if residues can be found at the different concentrations when applied (once or twice) during the honey flow.

Methods and Materials

Experimental Colony Selection

Sixty honey bee colonies were selected for the study from 79 analysed honeybee colonies in 4 separate apiaries. Honey supers were added as needed throughout the season to prevent swarming. In mid-June, weak hives were given pollen patties. The hives were selected based on colony strength, which was determined by the percentage of bees, the square inches of brood, and percentage of honey on each side of each frame in the colony. The sum of the brood on all frames in the hive were added together, resulting in total square inches of brood per hive. The mean percentage of bees per frame was calculated, and the frame means were then averaged to produce the mean percentage of bees, per frame, in the hive. The same procedure was used to determine the mean percent honey in each hive. As hives were being assessed, they

were ranked based on qualitative strength; one being the weakest hives and five being hives about to swarm. Only hives ranked three or higher were used in the experiment. Dead hives were removed from the experiment and queen-less hives were documented and the queens were replaced. Data was not used from hives that had abnormal queen losses.

After all colonies were assessed, 19 hives were removed based on queens and strength. Next, the remaining hives were tested for *Nosema apis* and *Nosema ceranae* disease levels according to Reuter et al. Only hives with similar *Nosema spp.* levels were used in the experiment.

Each of the 79 hives was also tested for the Varroa infestation level using the alcohol wash method mentioned above. Only hives with an infestation rate between 2%-10% were used in the experiment. Sixty hives ranked 3-5 with similar Nosema levels and a Varroa infestation between 2%-10% were then randomly selected to be in the study. Hive strength was equalised by ensuring each hive had a similar amount of capped brood cells. The experimental hives were randomly divided into ten groups of six hives each. Group 1 had only five hives because a few weeks into the experiment, one hive was found to be queen-less and therefore could not be used.

Treatment Application

Treatments were administered at the beginning of three treatment periods (Table 1). The first two treatment periods were 14 days each, and the third and final treatment was 6 weeks long. Treatments were applied on May 29, June 16, and June 30. Single treatments of HopGuard II™ at concentrations of 21g/strip, 23g/strip, and 25g/strip were compared to two treatments of HopGuard II™ at the same concentrations (Table 1). Single and double treatments of Apivar® were used as a positive control, while no treatment

besides the final treatment was used as a negative control (Table 1). All hives were given a finishing treatment of Apivar®. The finishing treatment in hives which received double doses of Apivar® was removed on August 10.

Table 1. Experimental design comparing the efficacy of: one HopGuard II™ treatment, two HopGuard II™ treatments, one Apivar® treatment, two Apivar® treatments, and no treatment. Group 1 had only 5 hives because one hive was found to be queen-less, and had no brood, larvae, or eggs.

Number of Colonies	Graph Legend	Treatment Period		
		1	2	3
5	OneHop25	HopGuard II™ 25g/strip	Apivar	None
6	OneHop23	HopGuard II™ 23g/strip	Apivar	None
6	OneHop21	HopGuard II™ 21g/strip	Apivar	None
6	EarlyApi	No Treatment	Apivar	Apivar
6	Apivar	Apivar	No Treatment	Apivar
6	TwoHop25	HopGuard II™ 25g/strip	HopGuard™ 25g/strip	Apivar
6	TwoHop23	HopGuard II™ 23g/strip	HopGuard™ 23g/strip	Apivar
6	TwoHop21	HopGuard II™ 21g/strip	HopGuard™ 21g/strip	Apivar
6	LateApiv	No Treatment	No Treatment	Apivar
6	Apivar	Apivar	Apivar	Apivar

To ensure the cardboard strips were absorbing different amounts of HopGuard II™ at each concentration, the contents of 1 package of each concentration of HopGuard II™ was weighed before and after the HopGuard II™ was added to the hive. The amount of liquid HopGuard II™ not absorbed by the cardboard was used to determine which strips absorbed the most HopGuard II™.

Strips were placed as the manufacturer recommended with 1 strip of HopGuard II™ for every 5 frames of bees. Two strips were placed in the centre of the brood cluster in each brood chamber, with at least one frame between the strips. The front entrance and bottom boards of hives treated with HopGuard II™ ™ were checked for two days after the treatment was applied to determine if the HopGuard II™ causes mass bee die-off. When Apivar was added to a hive, two strips (per 5 frames of bees) were suspended from toothpicks and hung in the centre of the bee cluster in the brood chamber, at least two frames apart.

Data Collection

The colonies were scored in terms of the square inches of brood, the percent of bees on each side of each frame, and the percent of honey on each side of each frame. Colonies were scored on June 15th, June 29th, July 13th, July 27th, and August 10th. *Nosema spp.* and Varroa samples were taken on June 1st, June 29th, and August 10th. Sticky traps were placed in the bottom of the hives to determine the number of mites being killed by the treatment groups. The traps were trimmed to 165 square inches and changed periodically through the treatment period (Days 1, 2, 3, 5, 8, 11, and 15). When the sticky traps were collected, they were covered with plastic wrap and stored in a freezer to prevent mould growth. The number of mites on each sticky trap was counted twice, and the average of the two counts was determined. Honey and wax samples were taken at the start of the first treatment period, and at the end of the third treatment period. These samples were sent for testing to determine if HopGuard II™ accumulates in honey and wax. Efficacy of the various treatments was calculated using the formula:

$$\text{Efficacy} = \frac{\text{mites killed by treatment}}{\text{mites killed by treatment} + \text{mites killed by finishing treatment}} \times 100$$

The analysis of beta acids in honey was performed using a slightly modified version of a method developed for the determination of pesticide residues in honey (Paradis et. al. 2014). This method is based upon the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) method which has been widely adopted for the determination of pesticide residues in various food commodities (Anastassiades et. al. 2003). In the procedure used for the determination of beta acid residues in honey, the sample (5 grams) is dissolved in 10 mL of reagent water. Acetonitrile (10 mL) is added to the dissolved honey sample followed by a mixture of QuEChERS salts (magnesium sulfate, sodium chloride, sodium citrate dibasic sesquihydrate, and sodium citrate tribasic dehydrate). The mixture is shaken and then centrifuged. The QuEChERS salts facilitate the separation of water and acetonitrile. A 3 mL aliquot of the acetonitrile layer (which contains the beta acids) is transferred to a centrifuge tube containing 0.5 grams of octadecylsilane (C18) adsorbent. After shaking the tube is centrifuged and an aliquot of the cleaned supernatant is transferred to a vial for analysis by liquid chromatography-tandem quadrupole mass spectrometry (LC-MS/MS). The chromatographic separation in the LC-MS/MS analysis was achieved using a C18 LC column using a gradient elution program with methanol and water as the mobile phases (both containing 5 millimolar ammonium formate and 0.1% v/v formic acid). The MS/MS was operated in the negative ion electrospray ionization mode using multiple reaction monitoring for the detection of the target analytes. The analytes included in the analysis were the beta acids adlupulone, lupulone, and colupulone. The beta acid standard reference material used for quantitation of the residues was the International Calibration Extract 3 (ICE-3) for the HPLC analysis of alpha and beta acids (American Society of Brewing Chemists). Adlupulone and lupulone were quantitated together as a single residue because only the summed concentration of these two analytes is provided for the reference material.

Results

There was no significant difference between 2 applications of HopGuard II™ and strip concentration; the efficacy for 21g, 23g and 25g per strip were 64%, 63% and 62% respectively (Fig. 1). The efficacy of a single application of HopGuard II™ at 21g and 23g per strip was not significantly different from the control (42%, 38% and 29%;), (Fig. 1). However, two applications of the three concentrations showed good efficacy. A second application of HopGuard II™ strips increased the efficacy of the treatment to from 49% at 25g per strip, 42% at 23g per strip and 38% at 21g per strip to 64%, 63% and 62% respectively.

There was no difference in honey bee population between the different HopGuard II™ treatment applications regardless of concentration or number of applications (Fig. 2.). Of the 26 colonies treated with HopGuard II™, 65% of the extracted honey samples had beta acid residues detected (Table 2.), whereas 88% of the brood chamber honey had residues detected.

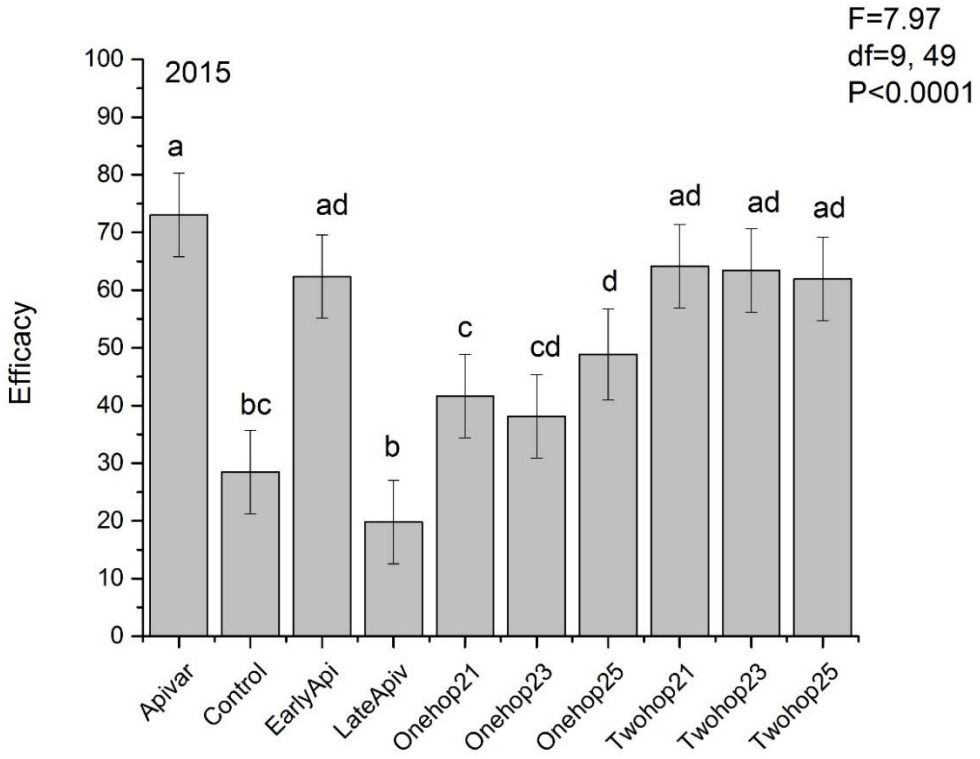

Fig. 1. Efficacy of HopGuard II™ treatments.

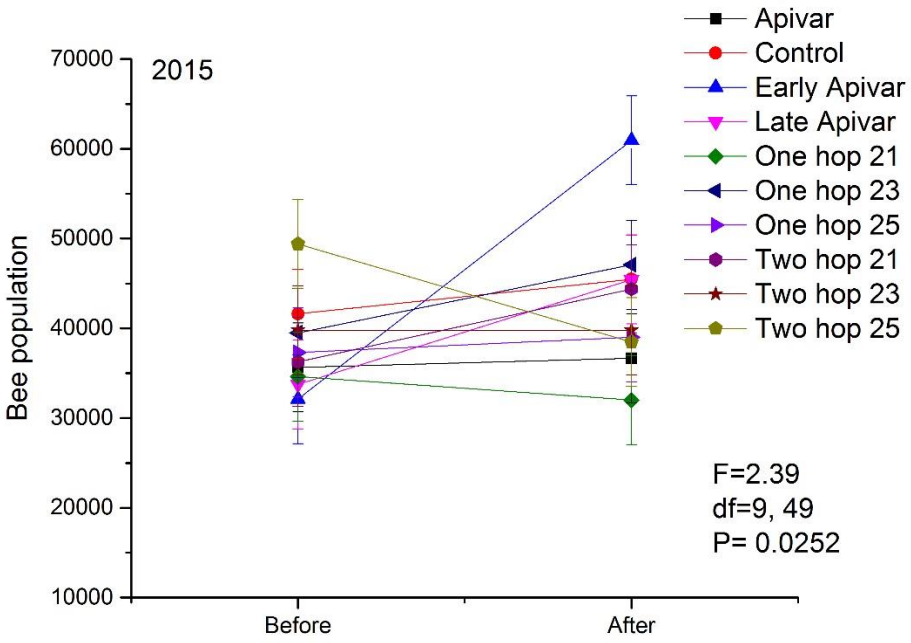

Fig. 2. Honey bee population before and after treatment.

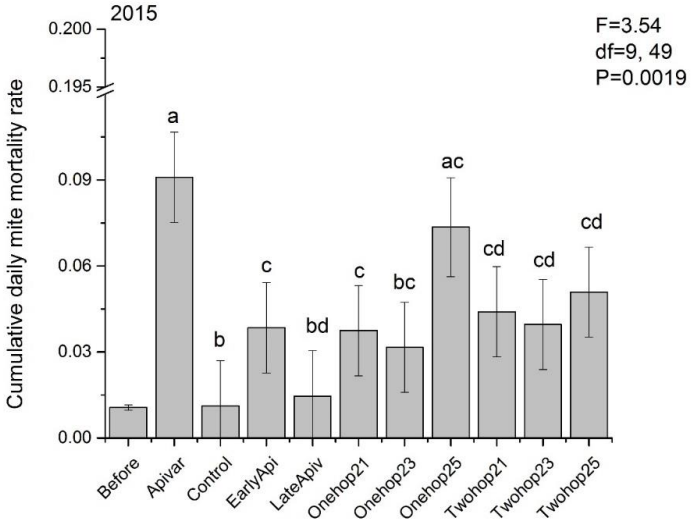

Fig. 3. Cumulative daily mite mortality for HopGuard II™.

Table 2. Analyzed Beta acid residues in brood honey and extracted honey.

Extracted Honey Samples

Treatment	Number of samples analyzed	Number of samples with residues detected	Average level of Beta Acid residues (ng/g)
HopGuard II™ 21grams applied twice	4	2	7.5
HopGuard II™ 21 grams applied once	4	2	2.9
HopGuard II™ 23grams applied twice	6	5	21
HopGuard II™ 23 grams applied once	5	4	4.8
HopGuard II™ 25grams applied twice	3	2	4.6
HopGuard II™ 25 grams applied once	4	2	9.5
Total	26	17	8.4

Brood Honey Samples

Treatment	Number of samples analyzed	Number of samples with residues detected	Average level of Beta Acid residues (ng/g)
HopGuard II™ 21grams applied twice	4	4	96
HopGuard II™ 21 grams applied once	4	1	13.3
HopGuard II™ 23grams applied twice	6	6	56
HopGuard II™ 23 grams applied once	5	5	49.5
HopGuard II™ 25grams applied twice	3	3	75.6
HopGuard II™ 25 grams applied once	4	4	66
Total	26	23	59.4

Discussion

Although Beta Acid residues were detected in all of the extracted honey samples, it is generally recognized as safe (GRAS) for human consumption according to the United States Food and Drug Administration Agency and has been used in food items such as hotdog casings as well as animal feed due to its antimicrobial properties (FDA, 2001).

A second application of HopGuard II™ strips significantly increased the efficacy of the treatment supporting the past experiments. Although two applications is better at controlling colony mite levels than one application, 64% is poor efficacy for a miticide. The HopGuard II™ label states that the strip can be applied in the summer. However, HopGuard II™'s ability to effectively decrease mite populations below the economic threshold during the summer fails to be substantiated by these results.

Experiment 5. HopGuard II™ dosage and application, Spring 2016.

Objectives

Since the loss of Apistan® and Checkmite® as effective miticides, Alberta Agriculture and Forestry has been screening new and potential miticides to see how effective they are under Alberta's conditions. Previously the effects of HopGuard II™ on mite levels, brood and bee production have been examined under summer and fall conditions. In this experiment HopGuard II™'s efficacy was tested under Alberta's spring conditions. The effect of dosage and number of applications was examined to determine its effect on bee and brood development as well as how effective HopGuard II™ is at decreasing mite levels in honey bee colonies. Generally hives in the spring have a small amount of brood present as the bees are just starting to build up after winter.

Methods and Materials

In the spring of 2016, colonies at Crop Diversification Centre North, Edmonton were used to test the efficacy of HopGuard II™ on reducing mite populations as well as the effect doubling the dose has on colony build up. To do this, 40 colonies were examined on April 28, 2016 to determine: mite infestation levels, if the colony had a queen, brood population, bee population, and amount of honey. 35 of the 40 colonies were then chosen to receive one of the following 5 treatments (7 colonies per treatment):

1. 1 strip of HopGuard II™ (HopRD) per 5 frames of bees applied 2 times, 10 days apart.
2. 2 strips of HopGuard II™ (HopDD) per 5 frames of bees applied 2 times, 10 days apart.
3. 1 strip of HopGuard II™ (HopRS) per 5 frames of bees applied once.
4. 2 strips of HopGuard II™ (HopSD) per 5 frames of bees applied once.
5. No treatment (Control)

Colonies received their first treatment on May 6th. At the same time approximately 300 bees were taken from brood frames and approximately 100 bees from the inner cover to determine *Varroa* mite infestation levels as well as *Nosema* spp. levels within each colony. Sticky traps were placed in all experimental

colonies on May 3 and taken out May 6 to get an initial daily mite drop. Once treatments were placed inside the colonies, sticky traps were changed every day for the first 3 days then on day 5 and on day 10. The same sticky trap schedule was followed when the second treatment was placed in the colonies dependent on treatment group. Colonies that received only a single application of HopGuard II™ had Apivar® strips as their second treatment. This occurred on May 16, 2016. On May 27, experimental colonies which received a double application of HopGuard II™ or no treatment had Apivar strips (1 strip per 5 frames of bees) placed in the top and bottom brood chambers of the colonies. Sticky traps at this point were changed every day for the first 3 days, and then only on day 5, 10, and every 7 days following until the Apivar strips had been in the colonies for 42 days (the label recommendation). On May 3, May 24, June 7, June 22 and July 14 approximately 300 bees from the brood chamber and 100 bees from the inner cover were once again taken to determine the Varroa mite infestation and Nosema levels. Bees, brood and honey production were measured throughout the experiment on May 16, May 24, June 8, June 21, and July 4, 2016 to determine what effect, if any, HopGuard II™ had on the colony. This was assessed as a percentage of bees and honey on each side of the frame as well as the square inches of brood on the frame. The amount of brood was determined by taking a picture of each frame of brood and then analysing each using Image J (National Institute of Health) to determine the square inches of brood per colony. The mean percentage of bees per frame was calculated, and the frame means were then averaged to produce the mean percentage of bees in the hive. The same procedure was used to determine the mean percent honey in each hive.

Results

HopGuard II™ single applications showed low efficacy at the recommended dose and double the recommended dose 29%, 37% respectively, (Fig. 1). Two applications of the recommended dose and twice the recommended dose showed an efficacy of 62% and 65% respectively (Fig.1.). This was significantly higher than the single application of the same dosages. There was no significant difference between colonies that had 1 application of either the regular dose or double the dose. The same was found between the regular dose and double dose when applied twice.

Double dosing colonies with HopGuard II™ had no significant effect on brood development, or bee population when compared to the recommended dose and no treatment (control) colonies (Fig. 2. and 3.).

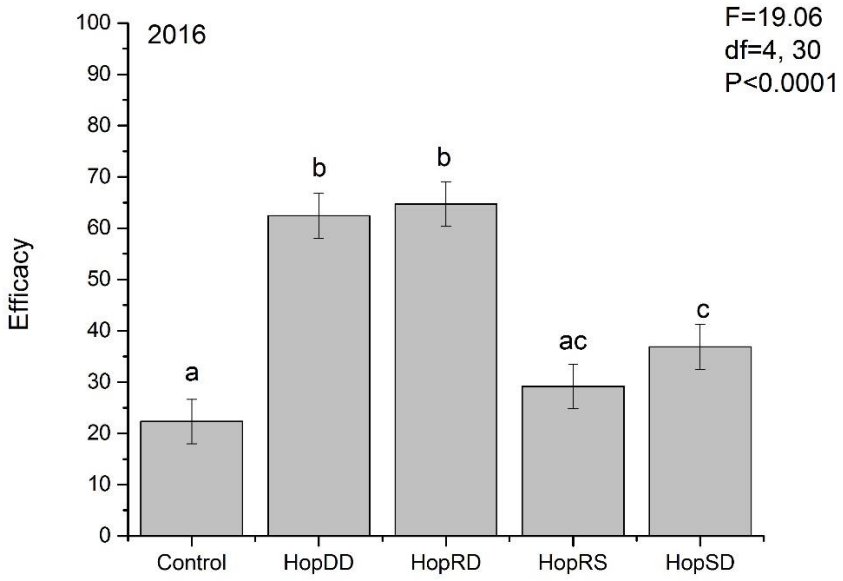

Fig. 1. Efficacy of HopGuard II™ based on dosage and number of applications.

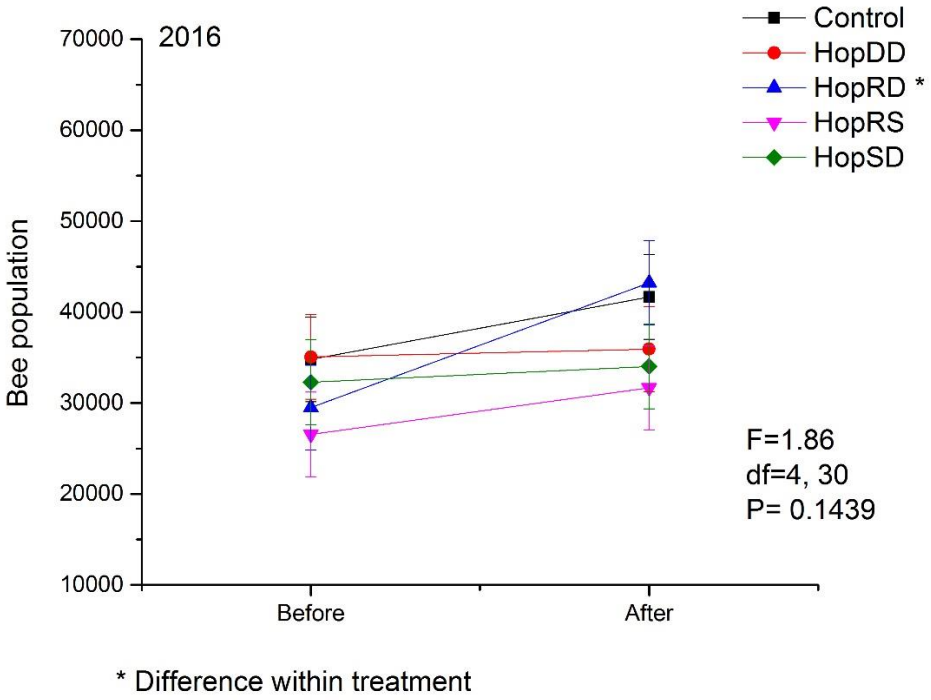

Fig. 2. Honey bee population before and after HopGuard II™ treatments.

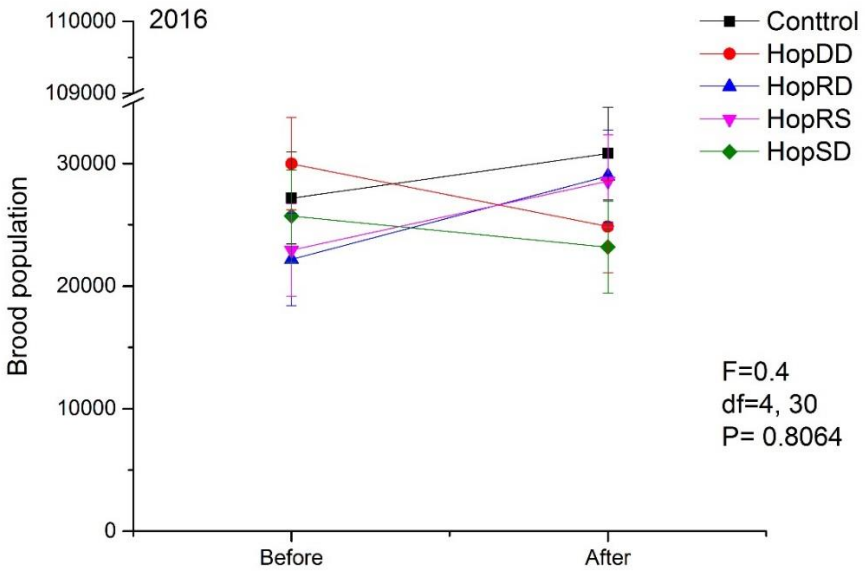

Fig. 3. Area of brood before and after HopGuard II™ treatments.

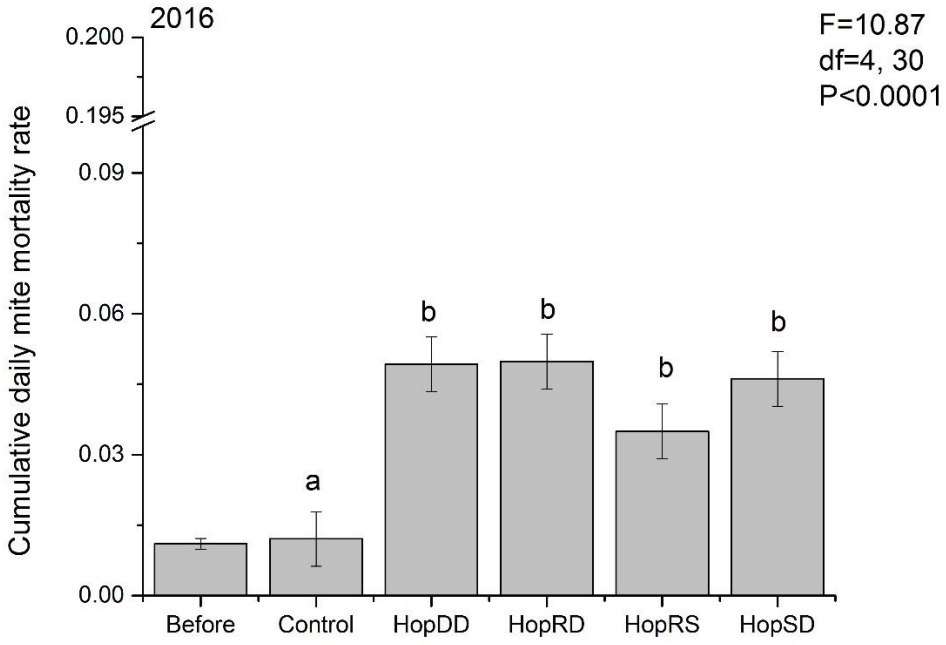

Fig. 4. Cumulative daily mite mortality based on dosage and number of applications.

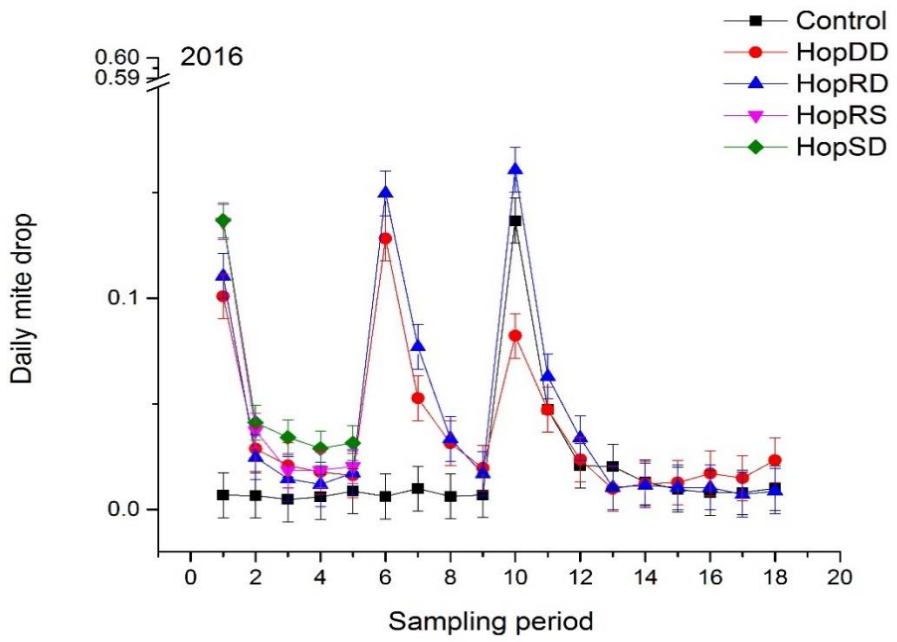

Fig. 5. Daily mite drop based on dosage and number of applications.

Discussion

It is felt that due to abnormally early spring Alberta experienced in 2016, the bees at the start of the experiment were approximately 2-3 weeks ahead of what would be expected under average Alberta conditions. As a result, there was more brood present in colonies then would normally be expected at the time of the year. This would account for the lower efficacy HopGuard II™ had on Varroa mite populations. It does however call into question the claim that HopGuard II™ can be used to decrease mite populations when there is a honey flow just as the previous experiment discovered. The results draw doubt on to whether HopGuard II™ can be effective as a miticide when brood is present inside the colony. It could however be effective at maintaining the mite population at a lower level consequently minimizing the damage a growing population of mites will have on the colony and its development.

It has been heard anecdotally that HopGuard II™ kills bees and brood. Our results do not support this claim. It is thought that perhaps applicators of HopGuard II™ may be pouring excess liquid in the HopGuard™ package (not absorbed into the strips) directly into the colony. This would most likely be responsible for bee and brood kill in colonies where brood and bee kill was reported.

Based on these results, one could conclude that a single application of HopGuard II™ is ineffective as a miticide when applied to colonies containing brood. Additionally, a second application of the miticide is required to decrease mite levels. Furthermore HopGuard II™, if applied properly, has no significant effect on brood, bee population or honey production.

Conclusion

In general based on these experiments it can be concluded that 21 grams of HBA in the new corrugated strips (HopGuard II™) is an effective mite control when applied twice 10 days apart in the fall. Treatments only done in the summer months during honey flow will show minimal mite control. Further experiments should be done to determine the efficacy of a third application of HopGuard II™ in the early summer, before the honey flow has begun. The recommended dose of 1 strip/ 5 frames of bees do not significantly affect brood and bee mortality. The highest mite mortality was found to occur in the first 48 hours of application after which HopGuard™ seems to become increasingly ineffective as a miticide.

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Oxalic acid – Comparison of Two New Application Methods of Oxalic Acid Solutions in two different Substrates in Late Fall Season

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Abstract

Two new methods of applying oxalic acid to honey bee colonies for the control of Varroa mites were evaluated in Alberta, Canada, in fall 2017. These methods were designed to prolong the exposure of bees to oxalic acid compared to sublimating oxalic acid into the colony, or drenching the bees with an oxalic acid/sugar solution. Colonies received one treatment of oxalic acid in cardboard strips (Maggi et al. 2015), or two treatments of Scott® blue shop towels (Oliver 2017), depending on treatment group. The efficacy of the tested methods was determined 36 days after treatment. Based on washing bee samples collected before and after the treatments to determine the mite infestation level, the efficacy of the cardboard strips and Scott® blue shop towels was 96.8%±3.9%, 85.6%±18.3%, respectively. The efficacy of Scott® blue shop towel method was very variable in treated colonies. There was also a significant difference between the daily mite drop in the control, Scott® blue shop towel and cardboard strip treatment groups. Further analyses showed that there was no significant difference between the mite drop in the control and the blue shop towel treatment groups, but the chipboard strips had the highest mite drop through the application period. The low drop of mites in the shop towel group could be due to the placement of the towels in between the two brood chambers. We observed that mites dropping from the top brood chamber landed on the towels, resulting in inaccurate mite drop counts on the sticky traps. When Oxalic acid followed by Apivar as a finishing treatments, the efficacy of the cardboard strips and Scott® blue shop towels was

97.6%±3.7%, 82.1%±9.3%, respectively. Potential of further testing and developing these methods to meet Alberta conditions to be used by beekeepers in Varroa management system is discussed.

Introduction

Oxalic acid has proven to be effective as a miticide against Varroa mites when colonies are broodless. The effectiveness of killing mites ranged from 90% - 98% in broodless colonies (Charrière et al. 2001; Radedzki et al. 2001; Nanetti et al. 2003; Rademacher and Harz, 2006). For oxalic acid to be used successfully, conditions specific to each geographical location must be considered. Researchers reported variable efficacy levels of oxalic acid against Varroa mites depending on the interaction between application method, dosage, beekeeping management practices, bee and Varroa mite population dynamics, presence of brood, geographic locations, and climatic conditions (Rademacher and Harz, 2006).

There are three main methods of applying oxalic acid; the drip/trickle method, the spray method, and the sublimation/evaporation method. A summary of oxalic acid application methods and the pros and cons of each is presented in Table 1. The drip or trickle method is registered in Canada as a method of applying oxalic acid to honey bee colonies for Varroa control. It consists of dissolving 35 g of oxalic acid in one liter of sugar solution (50:50; weight: volume). The applicator then drips or trickles 5 ml of the solution onto the bees occupying the bee space between the frames, in both the top and bottom brood chamber. The recommended maximum dose is 50 ml per colony whether bees are in nuclei, single, or multiple brood chamber hives. The trickle application of oxalic acid must be done when outside temperatures are between 3-13ºC.

The efficacy of the dripping/ trickling treatment as a miticide varies depending on the concentration of oxalic acid in the solution, the size of the colony, the presence of brood in the colony, and the climate in which the application took place. The trickle method was found to be 95% effective at controlling Varroa mites when the colony was broodless (Mutinelli et al. 1997; Nasr et al. 2001; Charrière and Imdorf. 2002; Nanetti et al. 2003). In the case of double brood chamber bee colonies, the dose must be divided between the top and bottom brood boxes to ensure that all bees are exposed to oxalic acid.

When oxalic acid is applied only to the top box, the efficacy drops to approximately 65%. However, trickling the oxalic acid on bees in the bottom box and on the top box can increase the efficacy to 95% (Nasr et al. 2001).

Büchler (2000) estimated the dose per bee. He found that applying 22 ml of oxalic acid in sugar syrup to a small colony with 7400 bees, would dose each bee with 3 µl. The efficacy for this treatment using 3% oxalic acid solution was 91.3%. When the concentration was increased to 4.6%, the mite mortality increased to 98.4%. Additional research showed that higher doses do not necessary increase efficacy (Liebig, 1998; Charrière, 2001; Charrière and Imdorf, 2002; Nanetti et al., 2003).

The efficacy of trickling oxalic acid solution decreases to 36% when brood is present (Schuster and Schürzinger, 2003). Repeated treatment with 4.1-5.2% oxalic acid in sugar solution achieved 39-52% efficacy (Gregorc and Planinc 2001). Other studies reported similar results (Mutinelli et al., 1997; Baggio and Mutinelli, 2003b).

The spray method is similar to the drip/trickle method in that a specific concentration of oxalic acid sugar syrup solution is applied onto bees in the colony. This method is considerably more time consuming and invasive to the colony as the applicator is required to remove each frame with bees and spray both sides with 3-4ml of the solution (Johansson 1997). This method has proven to be 80%-99% effective at reducing the mite population in the colony when no brood was present (Charrière et al. 2004; Nanetti et al. 2006). In the presence of brood, only 60% of the mites were killed (Charrière et al., 1998). Trials with repeated treatments (every seven days for four weeks) were carried out when brood was present in the colonies resulting in mite mortality from 73% in spring and 94% in autumn, respectively (Higes et al., 1999).This method is not registered for use by Canadian beekeepers.

The sublimation method, also known as the evaporation method, involves heating oxalic acid powder until it is sublimated as a gas in the colony. The gas forms fine crystals inside the hive, which settle on the bees. This method is less invasive to the colony because there is no need to open the hive, thus, the bees are not disturbed or exposed to cold temperatures. The gas is simply administered through the hive entrance. This method can result in up to 97% of mites being eliminated when colonies are broodless (Al Toufailia et al. 2015). In Canada, it is recommended to place 2.0 g of oxalic acid dihydrate powder into a sublimation machine such as Varrox or the VarroaXX. The sublimation machine of Varrox or the tip of delivery tube of VarroaXX is then inserted inside the front entrance of the bottom brood chamber. After heating the hot plate containing the acid powder, oxalic Acid gas is released into the bee cluster to treat mites. Sublimation of oxalic acid can provide mite mortalities > 90%, but is less effective if brood is present (Nasr et al. 2007).

Table 1: Summary of Oxalic acid application methods.

Type of application	Method of application	Amount of acid applied	Frequency of application	Pros	Cons
Oxalic Solution in sugar syrup	Dripping / trickling	1.75g	Once	Cheap, less labour intensive then spraying	weather dependent, some bee and larvae deaths
	Spraying		Once	Cheap	Weather dependent, labour intensive, some bee and larvae deaths, invasive
Gas-Sublimation/ Evaporation	Passive sublimation (e.g. Varrox)	2 g	1 – 2 times	Require more time in between applications, less labour intensive, less invasive to colony, Cheaper than pressurized machines,	Not effective when temperatures are <5°C, respirator required for applicator, Power source required
	Air pressured sublimation (e.g. VarroaXX)	2 g	1 – 2 times	Fast application time, less labour intensive and invasive to colony	Equipment is expensive, respirator required for applicator, Power source required
	Air pressured Fast sublimation (e.g. Fogger)	unknown	unknown	Fast application time	Machine requires calibrating for accurate dose. Hasn't been properly tested respirator required for applicator, Power source and air blower required

Although all these methods have proven to be effective at controlling Varroa mites, their treatment times are limited solely to when there is no brood inside the colony. This greatly restricts the window at which treatments can be applied (Brodgaard et al. 1999; Rademacher and Harz 2006; Nasr et al. 2007). Current treatments are most effective when the colony is broodless because oxalic acid has little to no effect on mites that are developing in capped cells (Imdorf et al. 2003). Consequently, oxalic acid can only be used during a very short time window during the early spring or late fall/early winter, where brood is not present, or is minimal. This situation demonstrates that there is an urgent need for an application

method that prolongs the exposure of bees in a colony to oxalic acid for at least a full brood cycle in order to eliminate Varroa mites on bees when brood is present.

Researchers in Argentina created a new method of applying oxalic acid to the colony that will prolong the colonies exposure to the acid (Maggi et al. 2015). This method consists of soaking strips of cardboard in oxalic acid/glycerol solution (Table 2) and applying 4 U-shaped strips (45 cm×3 cm×1.5 mm) per single brood chamber colony. Strips were left in the colony for 42 days. Colonies received treatments in the summer (January to April) and autumn (May to June) months, when brood was present in the colony, in Argentina at 3 different locations. The average efficacy of the strips was 94% and 92.8% in the two summer locations and 92.8% in the autumn location (Maggi et al. 2015). Maggi et al. (2015) also found that this application did not increase oxalic acid content in honey and bees wax or cause negative effects to colony population.

Oliver (2017a) modified the Argentinian method by soaking the glycerol, oxalic acid mixture with the addition of water in Scott® blue shop towels (Table 2). One shop towel was placed between two brood chambers over the center of the cluster and left in the colony for 48 days. Oliver (2017a) suggested that this method would take less time to apply than the Argentinian method, as the applicator is only placing the towel in between the two brood chambers as opposed to placing 4 strips throughout the colony. The Scott® blue shop towels are also inexpensive and easy to find at most hardware stores.

Oliver (2017b) reported that the Scott® blue shop oxalic treatment reduced the rate of mite buildup by midpoint grading and by end point after 48 days of treatments resulting in 94% reduced the median mite count. Oliver also stated in an update that at three weeks into the trial, mite suppression was erratic. It also appears that the towel application method may not be adequate for mite control early in the season.

The results of the cardboard strip and Scott® blue towel methods cannot be compared because of the lack of clarity and evolving protocol when Scott® blue towel method was tested. Moreover, there was no direct comparison between the two methods to provide better understanding of the efficacies and side effects on bees. Therefore, the objective of our study is to compare the efficacy of cardboard strip method as described by (Maggi M. et al. 2015) and Scott® blue towel treatment method as described by Oliver (2017a) under Alberta's conditions in the fall of 2017.

Materials and Methods

All colonies were situated at Crop Diversification Centre North in Edmonton, Alberta, Canada. The experiment consisted of 22, double brood chamber Langstroth colonies. Standard management methods were used to manage the experimental colonies. Pre-treatment assessments were performed to determine Varroa mite infestation and mortality per day, bee population, and brood areas in each experimental colony.

Colony bee and brood evaluations.

The bee population and brood area were assessed in all experimental colonies. To assess bee population, colonies were scored visually by estimating the percentage of each side of each frame that was covered in bees. A full frame covered with bees had approximately 2430 bees based on reported results by Burgett and Burikam (1985). Brood area was assessed by photographing both sides of every brood frame. The area of capped brood was determined using ImageJ software (Schneider et al. 2012).

Determination of Varroa mite infestation

Washing samples of bees

A sample of approximately 300 bees was collected from each colony, and stored in jars containing 70% ethanol. The prevalence of Varroa mites in the samples was determined using the alcohol wash method (De Jong et al. 1982). The samples were agitated on an orbital shaker at 300 rpm for 10 min. Each bee sample was placed in a 12 mesh strainer and rinsed under running tap water. Varroa mites fell through the strainer and were collected in an 11L Rubbermaid® basin. The number of mites was counted and the bees left in the strainer were weighed to determine the weight of bees in each sample. The number of bees in each sample was calculated using the average weight of a single honey bee as determined by weighing three samples of 10 honey bees per sampling date.

Sampling bees to determine mite infestation levels was done before treatments of bee colonies to find initial mite infestation on October 10. Five samples were collected throughout the experimental period; after the treatment was finished but before colonies were sublimated with Oxalic acid as a finishing treatment on October 24, on November 14 before colonies received Apivar as a second finishing treatment, on November 24, and finally on December 15, 23 days after Apivar had been in the hives. After washing the samples, the number of Varroa mites found in a sample of bees was then divided by the number of bees in the sample and multiplied by 100 to determine the Varroa infestation level.

$$\text{Mite infestation \%} = \frac{\text{Number of mites}}{\text{Number of bees}} \times 100$$

Monitoring daily mite mortality using sticky traps.

To monitor the mite mortality per day, a piece of sticky trap (30 X 43 cm, Contech Inc., BC, Canada) was placed on the bottom board of each experimental hive to collect dying mites that fell through the screen. Colonies had sticky boards placed in them on October 6, 2017 for 3 days to get an initial mite

drop. Sticky traps were replaced, and the treatments began on October 10, 2017. Sticky traps were replaced on day 1, 2, 3, 5, and then every 7 days following treatment. A second treatment of shop towels was applied to colonies on October 24, 2017. Sticky traps were again replaced on day 1, 2, 3, 5, and every 7 days following treatment.

On November 14, colonies were sublimated with 2 grams of oxalic acid as a finishing treatment. Sticky traps were replaced at day 1, 2, 3, 5 and then every 7 days to collect fallen mites. Because the oxalic treatment had little effect on mite drop in any of the treatment groups, Apivar® was applied as a second finishing treatment (1 strip of Apivar® for every 5 frames covered with bees) on November 22, 2017. Sticky traps were replaced when the treatment was applied, and 1,2,3,5, 7, 14, and 21 days after treatment. Due to weather conditions, the experiment was forced to end early, and Apivar® and stick traps were removed from the colonies after 23 days. All removed sticky traps were covered with a thin film of plastic wrap and brought to the laboratory to count mites. Varroa mite mortality per day was calculated by dividing the number of mites found on the sticky trap by the number of days the sticky trap was kept in the hive.

Preparation of oxalic acid solution and soaking tested substrate materials

The method described by Maggi et al. (2015) was used to prepare the cardboard strips. Glycerin was heated to 60°C, while being continuously stirred with a magnetic stirrer on a hotplate. Once the glycerin reached 60°C, oxalic acid was added to make a solution of 10 g oxalic acid in 20 mL of glycerin for each strip (20 cm×3 cm×1.5 mm). The temperature was maintained while continuously stirring the solution until the oxalic acid was completely dissolved, which was determined to be when the solution went from opaque to clear. The solution was then poured over the cardboard strips placed in a plastic tray where it was allowed to be absorbed (Fig. 1). The same method was used with the shop towels with the addition of water to the glycerin solution before heating began (Fig 2). The ratio of oxalic acid: glycerin: water is

presented in Table 2. All prepared strips and blue shop towels were then air dried at room temperature under a fume hood.

Colony treatment procedure

The 22 hives in the experiment had a mite infestation level of 5 to 43%, with an average mite infestation level of 17.8%. The colonies were split into 3 groups, using a spilt plot design, based on mite infestation levels and number of frames covered with bees. 8 colonies were assigned to the cardboard strip treatment group. The prepared strips were applied to assigned bee colonies (2 20cm-strip per 5 frames of bees) by placing the strips in between the frames of the bees cluster to allow bees to be exposed to the strips during the treatment period (Fig. Fig. 3). Seven colonies were assigned to the Scott® blue shop towel treatment group. The Scott® blue shop towels were placed flat in between the two brood chambers. In the first application one towel was applied per colony, but in the second application two towels were applied per colony (Fig. 4). The third group of colonies (6 colonies) were not treated and used as a control.

Statistical analysis

One colony from the strip treatment and one colony from the towel treatment were removed from the experiment as they died part way through the experiment. The mite infestation levels were calculated from samples of washed bees. Then the efficacy was calculated as:

$$\text{Efficacy} = \frac{\%\ \text{mites before treatment} - \%\ \text{mites after treatment}}{\%\ \text{mites before treatment}} \times 100$$

The rate of daily Varroa mite mortality was calculated using Martin (1998) equation. The efficacy of tested treatments, based on total mites collected on sticky traps was calculated according to the following formula:

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

The effects of treatments on mite mortality was analyzed by ANOVA using a repeated measures analysis of variance using an autoregressive heterogeneous covariance structure, with treatment as main effects, colonies as subjects, and day as a repeated measure. (PROC MIXED, SAS Institute Inc. 2011). Proportions were arcsine transformed prior to analysis to improve normality and homogeneity of variance (Snedecor and Cochran 1980). All data are presented as untransformed means. Where significant interactions were observed, treatment means were compared using Bonferroni adjustment. A before and after control impact (BACI) design was used to assess the effects of treatments on Varroa mite abundance through the experiment, where treatments were treated as main effects, and period was treated as repeated measures with colony as the subject (Stewart- Oaten et al. 1986).

Table 2. Summary of tested two application methods of oxalic acid and control.

Item	Blue Scott Shop Towels	Cardboard Strips	Control
Number of colonies	7	8	6
Dosage	First TRT One shop towel per colony Second TRT two shop towels per colony of 2-brrod chambers	2strips per 5 frames of bees - maximum 8 strips per colony of 2-brrod chambers	No Treatment
Number of treatments	2	1	0
oxalic per strip / towel	12 grams	5 grams	NA
Glycerin per strip / towel	13 ml	10 ml	NA
Water per strip / towel	10 ml	NA	NA
Size of strip / towel	26.5 x 28 cm	20.5 x 3.5 cm	NA
Total amount of applied oxalic acid per colony	36 g	20-40g	-
Finishing treatment 1	Oxalic Acid Sublimation (2g/hive)	Oxalic Acid Sublimation (2g/hive)	Oxalic Acid Sublimation (2g/hive)
Finishing treatment 2	1 strip of Apivar per 5 frames of bees	1 strip of Apivar per 5 frames of bees	1 strip of Apivar per 5 frames of bees

Results

This study was designed to compare the efficacy of applying oxalic acid in cardboard strips as described by (Maggi M. et al. 2015), and in Scott® blue towel as described by Oliver (2017a) under Alberta's conditions. The study was conducted in the fall of 2017. The mite infestation levels were not significantly different among tested treatments including the non-treated control at the start of the testing process. After 36 days of treatment, there were significant differences in the levels of mite infestation based on washing bee samples among tested treatments ($F= 13.93$, d.f. $=2, 16$, $p= 0.0003$) (Fig. 5). The efficacy of the cardboard strips, Scott® blue shop towel treatments and control was 96.8%±3.9%, 85.6%±18.3%, and -87.3%±123.2, respectively. Varroa mites increased in the control colonies.

There were also significant difference in the daily mite drop as collected on sticky traps for tested treatments ($F=13.65$, d.f. 42, 206, $p<0.0001$) (Fig. 6). The daily mite drop was not significantly different between the control and Scott® blue shop treatment. However, the daily mite drop was higher significantly ($p=0.0007$) when oxalic acid was applied in cardboard strips when compared to the control or Scott® blue shop towel method (Fig. 7). When the efficacy of tested treatments was calculated after using oxalic acid and Apivar as finishing treatments, the efficacy of oxalic acid applied in strips, oxalic acid applied in Scott® blue shop towel and control was 97.6%±3.7%, 82.1%±9.3%, and 68.2%±23.5%, respectively (Fig. 8). It is evident that the efficacy of oxalic acid applied in blue shop towels is lower and variable in comparison to oxalic acid in strips. As the experiment continued it was discovered that mites dying in the top brood chamber fell and accumulated on the blue towels that were placed in between the two brood boxes instead of the sticky traps (Fig. 9). Consequently, collected mites on the sticky traps in these colonies did not accurately represent all dead mites during the treatment period.

Discussion

On average both the chipboard strip and Scott® blue shop towel methods used for applying oxalic acid proved to be effective at controlling mites in late fall when colonies had minimal brood. When the efficacy was determined based on determining Varroa mites after washing a sample of 300 bees, chipboard strip and Scott® blue shop towel methods had an average efficacy of 96.8%±3.9%, 85.6%±18.3%, respectively. Using a finishing treatment, the average efficacy of chipboard strip and Scott blue shop towel methods was 97.6%±3.7%, 82.1%±9.3%, respectively. Finding the chipboard high efficacy in killing mites supports results reported by Maggi et al. (2015). In our experiment the application of Scott® blue shop towel had lower efficacy with higher variability than the chipboard strips. As the experiment continued it was discovered that mites dying in the top brood chamber fell and accumulated on the blue towels placed in between the two brood boxes instead of the sticky traps. Consequently, collected mites on the sticky

traps in these colonies did not really represent all dead mites during the treatment period and the sticky traps are then considered an inefficient way of measuring mite drop for the Scott® blue shop towel method. This may explain why there was no significant difference between the control and towels in terms of the daily mite drop. Despite this low mite drop, there was a significant difference in mite infestation in the towel vs control colonies after treatment (Fig.1). The infestation levels was lower in colonies received oxalic acid treated towels. Moreover, the high variability in the efficacy for Scott® blue shop towel method could be due to variable exposure of bees to the towels placed in between the two brood chambers in comparison to the strips placed in the cluster. These variations in efficacy of applying towels supports results reported by Oliver (2017a, b and c).

Our results showed that a finishing treatment of oxalic acid sublimation had a limited success on killing mites. When the treatment was applied to colonies, the ambient temperature was -13ºC and the Varroxx applicator took much longer to expel the material into the hives more than previously reported. These conditions during the application of oxalic acid could affect the amount of oxalic acid sublimated into the hive. Therefore, applying recommendations on the use of Varroaxx including the proper temperature range (0 to -4ºC) must be followed when used (Nasr et al. 2007). In order to address this problem, a second finishing treatment was applied using Apivar. Apivar application was effective in killing the rest of the mites.

In conclusion, these results are promising for developing a practical long term application of oxalic acid to control Varroa mites in bee colonies. Our evaluation of oxalic acid application as in Chipboard strips and Scott® blue shop towels as described by Maggi et al. (2015) and Oliver (2017 a, b and c) under Alberta showed that these methods have potential to control mites in the fall when minimal brood and no brood found in bee colonies. In our testing the chipboard strips provided higher efficacy with minimal variations among tested colonies in comparison to the blue shop towels. These results were only reached when tests were conducted on double chamber bee colonies. Further testing to check efficacy on single brood chamber

hives or doubles in spring season and early summer is needed. The side effects on honey bees through the season and residues in honey must be also evaluated. The number of applications and the duration of application must also be assessed. Once these data collected, there is potential for these two methods to be recommended for use by beekeepers and become a component of the integrated pest management system for Varroa mites control in Alberta.

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Fig. 1. Preparing Oxalic acid solution in chipboard strips.

Fig. 2. Preparing Oxalic acid solution in Scott Blue Shop Towels.

Fig. 3. Applying oxalic acid in chipboard strips in bee colonies (2 strips / 5 frames covered with bees)

Fig. 3. Applying oxalic acid in Scott Blue Shop towel to a bee colony (1 towel in between the two brood boxes). Second application 2 towels were applied in between the two boxes.

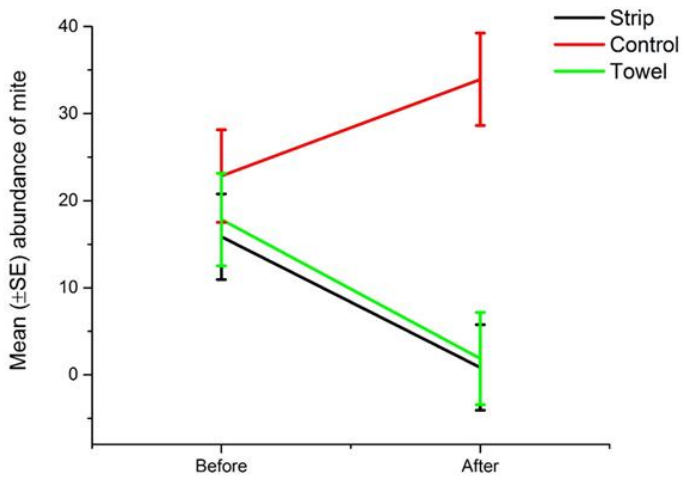

Fig. 5. Varroa mite infestation percentage before and after oxalic acid treatments. There were significant difference (F = 13.93, d.f. = 2, 16, p<0,0003).

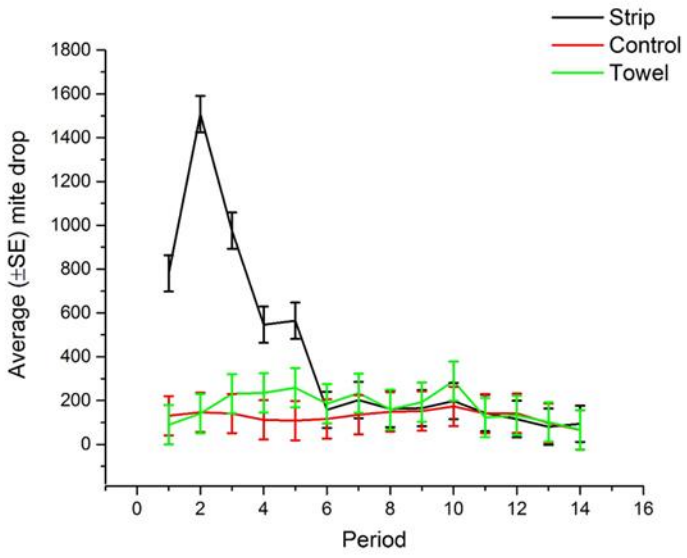

Fig. 6. Average daily mite drop on sticky traps for treatments. There were significant differences among treatments (F= 13.65, d.f. = 42, 206, p< 0.0001).

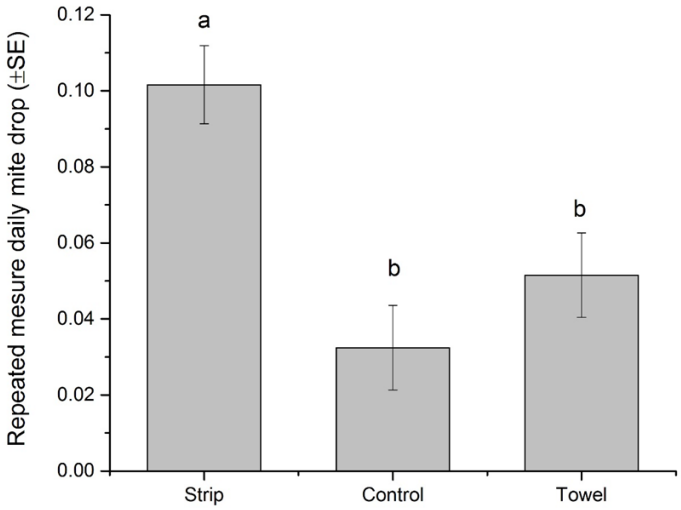

Fig. 7. Repeated measures of daily mite mortality for applied treatments and control. The daily mite mortality over monitoring period was significant.

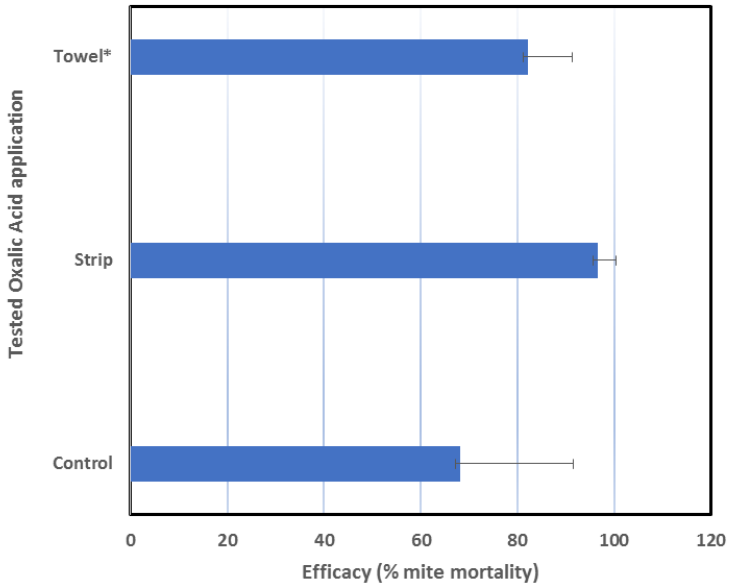

Fig. 8. Efficacy of tested treatments and control based on using oxalic acid and Apivar as a finishing treatment.

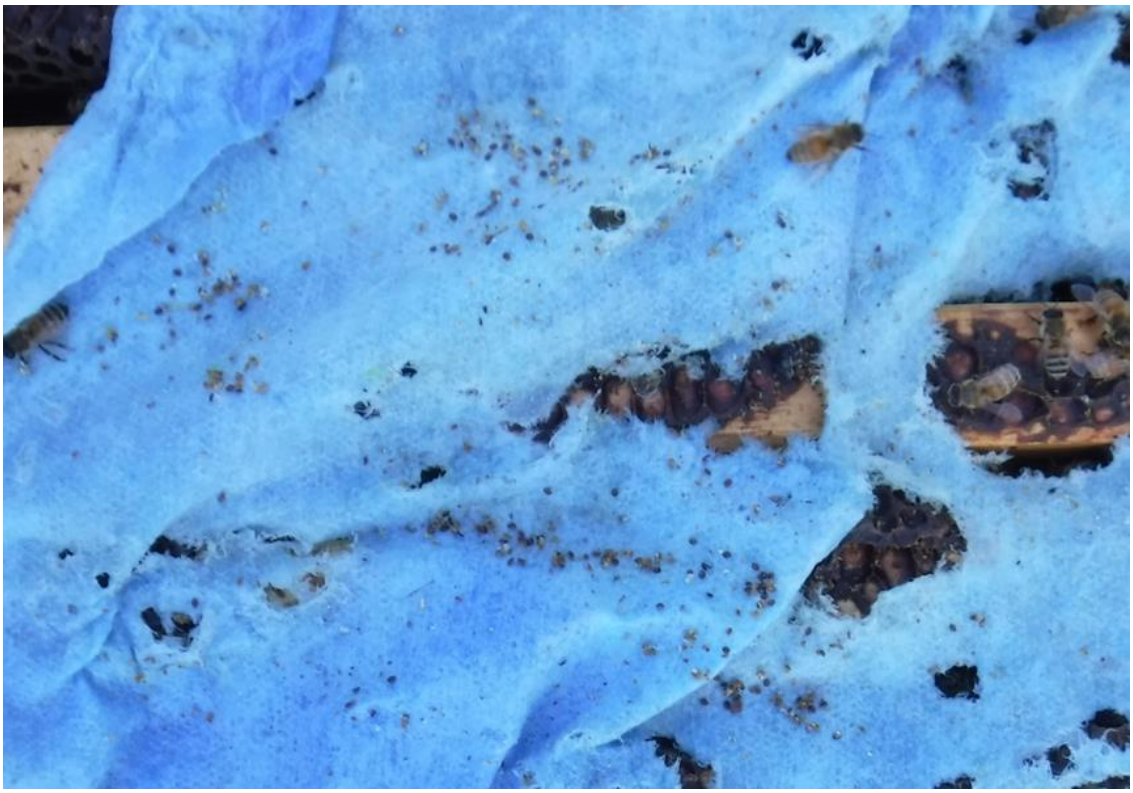

Figure 9. Towels placed in between the two brood chamber with Varroa mites dropped from the top brood box.

Assessment of *Nosema* epidemiology and control in Honey bees under Canadian Prairie conditions

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1. Introduction

Nosema apis (Zander, 1909) and *Nosema ceranae* (Fries et al., 1996) are obligate, fungal parasites that reproduce in the epithelial cell of the midgut of adult honey bees. The initial infection is caused when workers consume spores through either cleaning comb with infected feces or trophalaxis of contaminated food (van den Heever et al., 2014). Then the progeny either infect other midgut epithelial cells or are expelled from the body which spreads the infection. *Nosema* spp. reduces the lifespan of both infected and healthy honey bees (Higes et al., 2010). Due to their damaged midgut, infected honey bees are unable to obtain necessary nutrients and die of starvation. As infected workers die, the colony must compensate for this loss by encouraging early foraging in healthy bees. The energetic costs and risks of foraging can also result in a premature death for these otherwise healthy bees. All these effects can cumulate into a loss of honey production in infected colonies. Unfortunately, both species of *Nosema* do not invoke any obvious symptoms and can only be diagnosed by light microscopy and molecular techniques (Fries, 2010). Because of its effect on colony health and production, *Nosema* is a source of concern for Canadian beekeepers.

Understanding the seasonal trends of parasites allows for the appropriate timing of treatments to prevent outbreaks. *N. apis* has a well-established seasonal trend in honey bees with the highest infection in the spring, low summer infection, another smaller peak in the autumn, and a slow increase over the winter (Copley et al., 2012; Guzmán-Novoa et al., 2010). *N. ceranae* has highly variable seasonality, though a

peak in the spring has been seen in several studies (Emsen et al., 2016; Traver et al., 2012). Very few seasonality studies have been done in Alberta's unique climate of long, cold winters and short, hot summers. *N. ceranae* was originally found in the Eastern honey bee (*Apis cerana*) but has recently infected the Western honey bee (*Apis mellifera*) in Canada (Currie et al., 2010). Furthermore, two studies in 2008 and 2010 suggested that *N. ceranae* was replacing *N. apis* as the predominant species in Canada (Copley et al., 2012; Currie et al., 2010; Emsen et al., 2016). Fumagillin has been the only effective treatment available for *Nosema* since the 1950s (van den Heever et al., 2014). Based on the seasonality of *Nosema*, it is recommended to apply Fumagillin in the spring and fall. However, it has not been tested whether both treatments are effective at controlling *Nosema* proliferation and preventing colony mortality. Along with seasonality, there are other factors that can affect *Nosema* abundance.

Climate and wintering method vary across Alberta. In Alberta, there is a noticeable climatic difference between the North and the South. Southern Alberta has a longer summer season and milder winters than Northern Alberta, due to chinook winds. Wintering methods involve beekeepers choosing to either overwinter their colonies outdoors or indoors. Colonies that are wintered outdoors are wrapped in insulated winter wraps to protect them from the elements and trap heat, though bees can still exit the hive. Colonies that are wintered indoors are moved to a wintering facility that is climate controlled and dark to prevent bee flight. The inability of the bees to perform cleansing flights in both wintering methods may affect *Nosema* abundance in the spring (Retschnig et al., 2017). Desai and Currie (2016) found that *Nosema* abundance increased in colonies wintered outdoors but decreased in colonies wintered indoors, whereas Williams et al. (2010) found wintering method had no effect on N. ceranae abundance when treated with Fumagillin. Therefore, the potential effects of climate and wintering method should be considered.

Based on this information, two research projects have been created. The first project is an experiment to determine the effect of Fumagillin treatment timing on *Nosema* abundance and winter mortality for indoor and outdoor wintered colonies in Northern and Southern Alberta. It is hypothesized

that the fall treatment is the most effective at reducing *Nosema* abundance and winter mortality, whereas the spring treatment will have no effect. The second project is a study to determine the seasonal variation of *Nosema* spore abundance and species composition over a 12-month period. It is hypothesized that seasonal patterns can be established for both *Nosema ceranae* and *Nosema apis* in Alberta. Also, *Nosema ceranae* will be the predominant species year-round in Alberta. The following report details the experiment carried out during the 2017 field season and its preliminary results. The results of the study have yet to be processed.

2. Methods

2.1 Experiment Design

Northern and Southern Alberta climates were represented by two apiaries near Edmonton, AB and two apiaries near Brooks, AB, respectively. Wintering method was also represented in each location by having one apiary wintered indoors and one apiary wintered outdoors. The apiaries were named as follows: North Outdoor (NO), North Indoor (NI), South Outdoor (SO), and South Indoor (SI). Initially, all the colonies within an apiary were equalized. This was accomplished by collecting all available brood frames and sharing them equally among the colonies. All bees were shaken into a large screened-cage and distributed equally among the colonies using a 2 L scoop. All colonies were given new queens 1-2 days later. Screened entrances and sugar syrup were used to prevent the bees from returning to their original colony for 1-2 days. Since the colonies have recently been put into winter at the time of this report, there is no measurable effect of wintering method to seen at this time. Therefore, only apiary location will be interpreted.

2.2 Treatments

A split-plot design based on apiary was used to assign the colonies to one of the following treatments: Spring Only Treatment, Fall Only Treatment, Spring and Fall Treatment, Control (no treatment). Each treatment had 8 replicate colonies. An additional 8 control colonies were added for use in the seasonality experiment. Therefore, each apiary had 40 colonies for a grand total of 160 colonies. We

used the formulated product Fumagilin-B (Medivet, AB, Canada) for both spring and fall treatments. The spring treatment used the drenching method which is the preferred method by Alberta beekeepers in the spring. The treatment consisted of 250 mL of treated 1:1 sugar syrup that was poured onto the bees four times every 4th day post-treatment. The total amount of syrup applied was 1 liter and the cumulative dose was 120 mg of Fumagillin per colony over a 13-day period. The spring treatment was given to the NO and NI apiaries on June 30, July 4, 8, and 12, and to the SO and SI apiaries on July 6, 10, 14, and 18. The fall treatment used the bulk feeding method which is the preferred method by Alberta beekeepers in the fall where 3.7 liters of 2:1 sugar syrup containing 120 mg of Fumagillin was poured into the in-hive feeders. The fall treatment was given to the NO and NI apiaries on September 8 and to the SO and SI apiaries on September 6.

2.3 Sampling

Sampling for *Nosema* spp. and *Varroa destructor* were obtained once in June and biweekly from July to September. For *Nosema*, approximately 100 adult bees were collected from the outer honey frames in the brood chamber or honey supers. For *Varroa* mites, approximately 300 bees were collected from interior brood frames. The samples were obtained by sliding the sample jar with 70% alcohol gently down the length of the frame, causing the bees to fall into the jar. The colony was also visually inspected for other adult and brood diseases.

2.4 Evaluating colony health

The colony health assessment was based on measures of bee population, total brood area, stored honey, and pest and disease levels. This was evaluated in mid-July, mid-August and twice in September. Bee population was estimated by recording the approximate percentage of bees covering each side of the hive's frames. It was assumed that 2430 bees fully cover a frame (Burgett and Burikam, 1985). Frames containing brood were photographed and analyzed using ImageJ (National Institutes of Health, USA) to calculate the square inches of brood per colony. Colonies without brood diseases and pests (excluding *Nosema*) below the economic threshold were considered healthy. All colonies were treated with Apivar® (500 mg Amitraz/strip) in the fall to reduce *Varroa* populations for the winter. The amount of stored honey

was estimated by visually recording the approximate percentage of capped honey on each side of the hive's frames. Queen presence, supersedure, and acceptance as well as colony mortality and viability were recorded. Colony mortality was categorized as no live bees in the hive. Colonies were considered non-viable from a commercial stand point when there were less than 4 frames of bees in the colony in early spring and late fall.

2.5 Determining *Nosema* abundance

In order to obtain a preliminary estimate of possible treatment effects, the samples belonging to each treatment and apiary were pooled into a composite sample (see Figure 1). *Nosema* samples were prepared by grinding the abdomens of 30 bees with 5 mL of water in a 35 mL conical tissue grinder (VWR). An additional 10 mL of water was used to rinse the tube and grinder. Samples were analyzed using light microscopy and hemocytometer (adapted from Cantwell 1970). Samples were vortexed before analysis to ensure even distribution of spores. The samples were allowed to settle for 30 seconds after being loaded into the hemocytometer. Each sample was counted twice to produce a unit of million spores/bee. Samples from each colony are currently being processed for statistical analysis where the hive will be the experimental unit.

3. Results and Discussion

A total of 960 *Nosema* samples and 960 *Varroa* samples were collected from June to September 2017. Also, over 8000 photos of brood were taken. From this, a total of 96 composite *Nosema* samples were created. The following results are preliminary data from the composite *Nosema* samples, *Varroa* samples, and bee population estimates.

The average *Varroa* mite mean abundance by apiary stayed below 1.3% (1.3 mites per 100 bees) over the entire experiment (Figure 2). However, there were individual colonies in all apiaries, except NI, that were above the economic threshold of 3%, the highest being a 9.1% *Varroa* infection. Therefore, all colonies were treated at the beginning of September with Apivar. The treatment was effective at reducing

the average mite infection by apiary to below 0.5%, with the highest colony infection at 2.0%. In addition, mite infection levels were higher overall in the Southern apiaries than the Northern apiaries.

All figures show that *Nosema* infection was well above the 1 million spore economic threshold at the start of the experiment. In all cases, *Nosema* levels drop below the economic threshold by the beginning of August (Figure 3-8). Figure 3 shows the clearest trend with *Nosema* infection gradually decreasing for treated colonies whereas untreated colonies remained consistently high until a sudden drop at the beginning of August. This trend is seen in the Northern colonies (Figure 4) but not in the Southern colonies (Figure 5). The trend becomes progressively unclear as all treatments are shown and further divided by location (Figure 6-8). By analyzing the individual samples, these trends should become more clear. The highest *Nosema* infection was 11.05 million spores/bee in the July 25th in the NO Control composite sample.

Colonies began the experiment with between 12 000 and 15 000 bees or 5-6 frames. In all cases, treatment does not appear to have any effect on adult bee population as it increased by approximately 5000 bees from July to August. When they were last checked at the end of September before going into winter, colonies had approximately 20 000 bees or 8 frames.

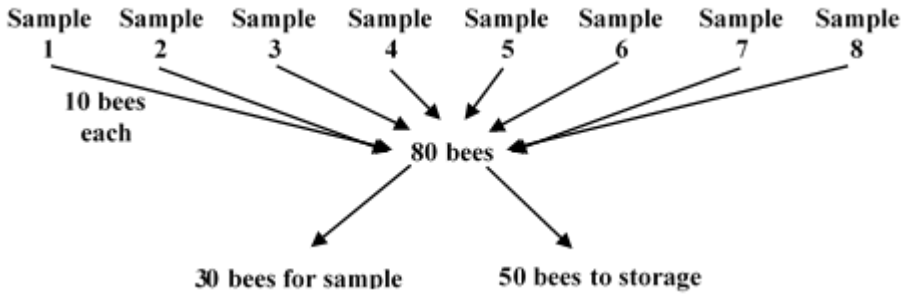

Figure 1: A diagram explaining the creation of the composite Nosema samples for each apiary and treatment.

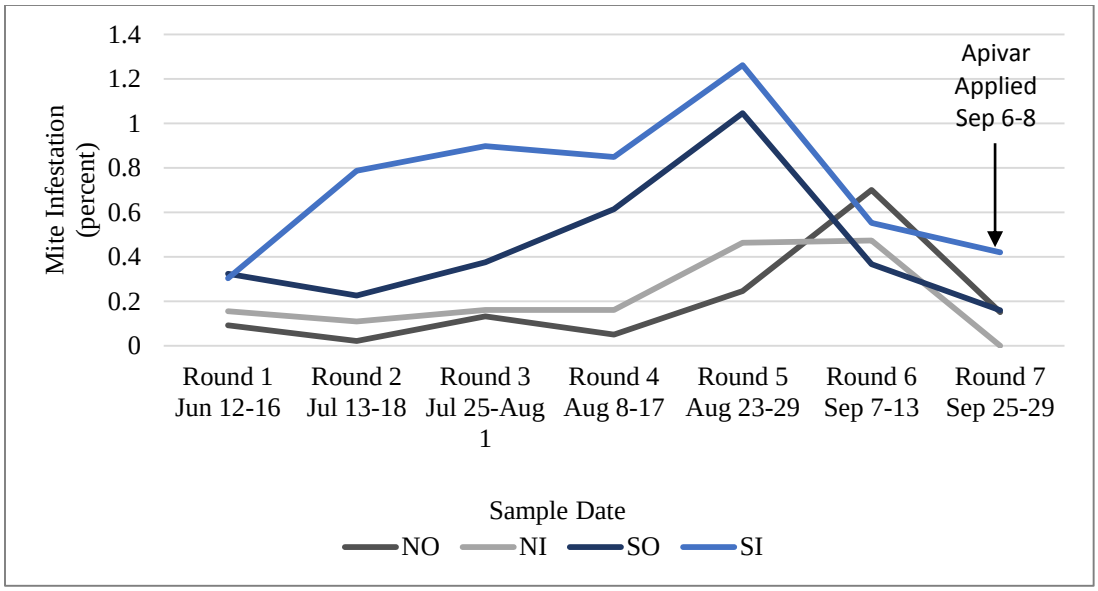

Figure 2: Average mite infestation by apiary.

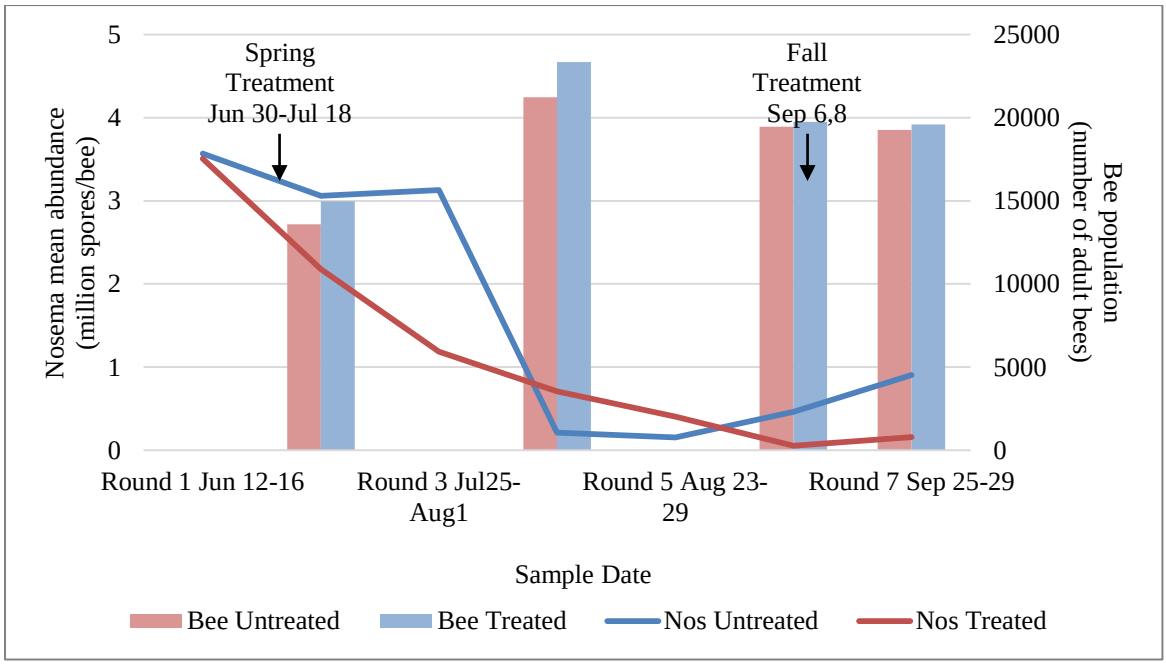

Figure 3: Change in *Nosema* mean abundance and bee population over time for colonies that were untreated or treated in the spring. For bee population, an average of n=55 and n=59 for untreated and treated, respectively.

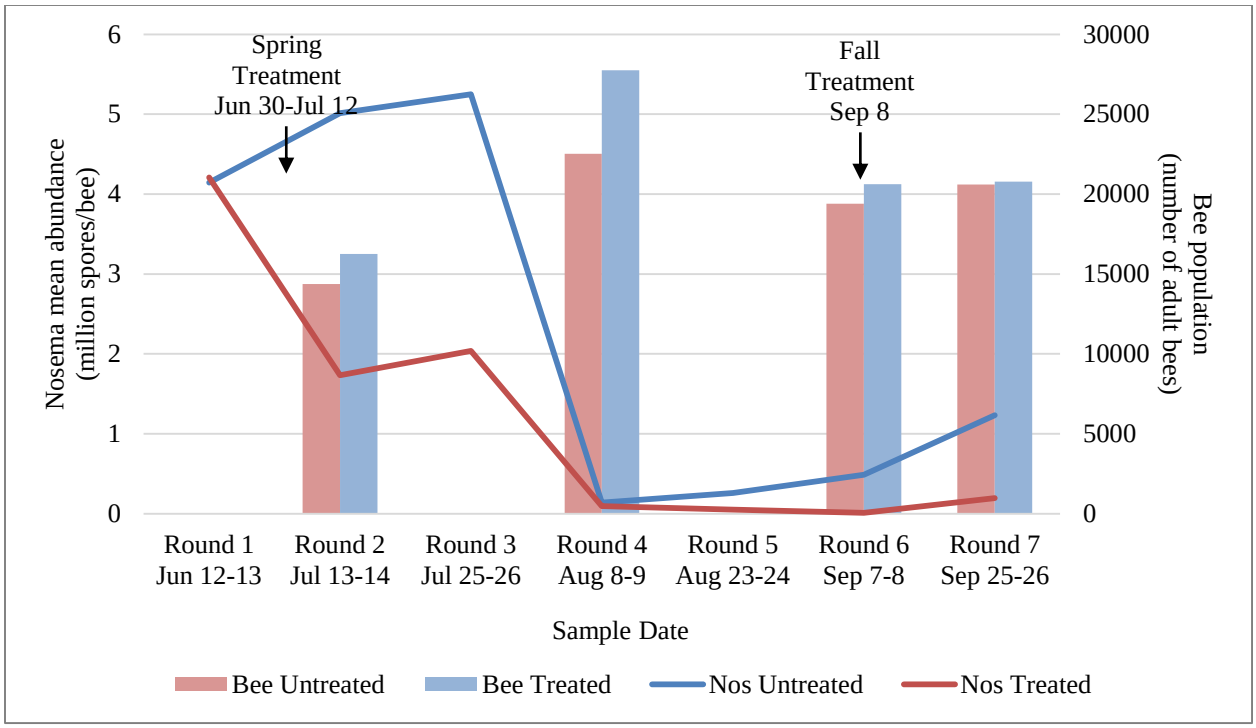

Figure 4: Change in *Nosema* mean abundance and bee population over time for colonies that were untreated or treated in the spring in Northern apiaries. For bee population, an average of n=29 and n=31 for untreated and treated, respectively.

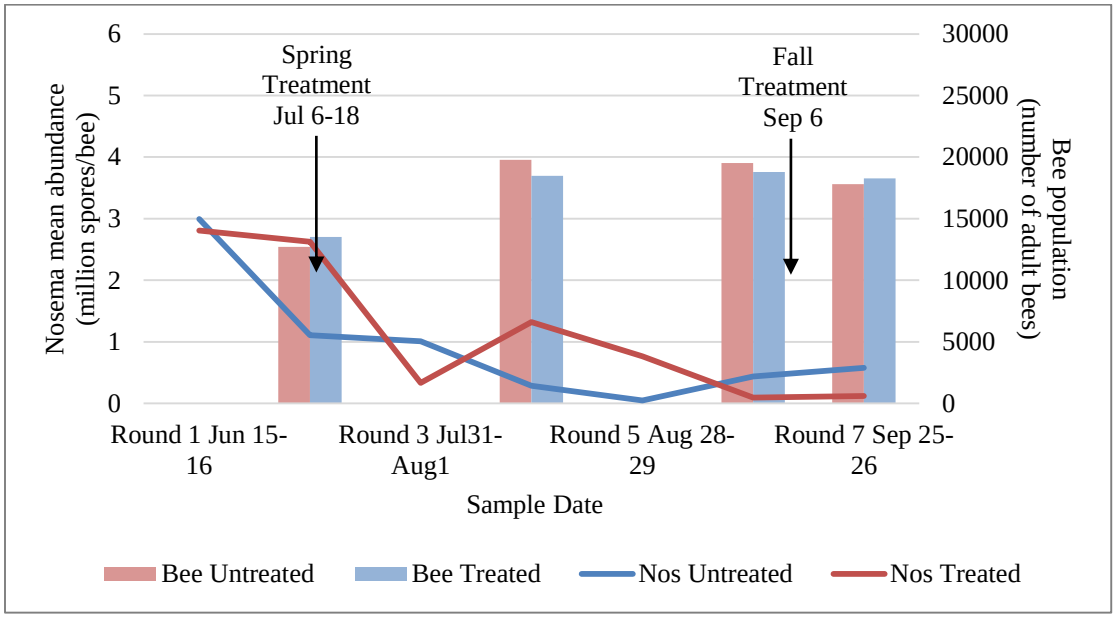

Figure 5: Change in *Nosema* mean abundance and bee population over time for colonies that were untreated or treated in the spring in Southern apiaries. For bee population, an average of n=26 and n=28 for untreated and treated, respectively.

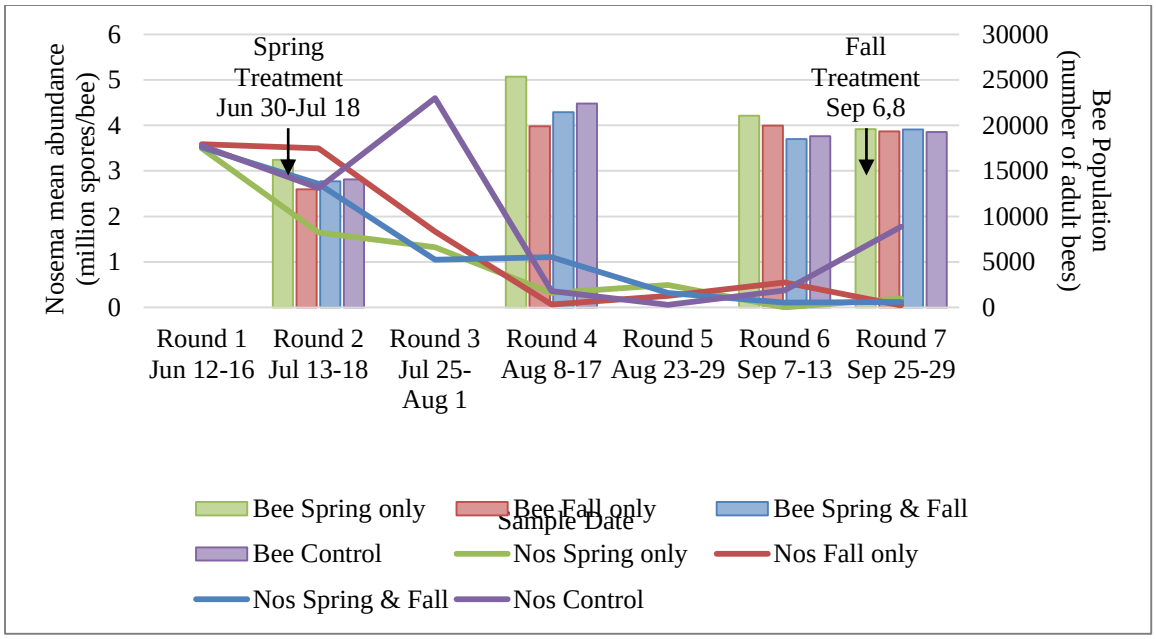

Figure 6: Change in *Nosema* mean abundance and bee population over time for all treatments. For bee population, an average of n=30 for spring only and n=28 for fall only, spring and fall, and control.

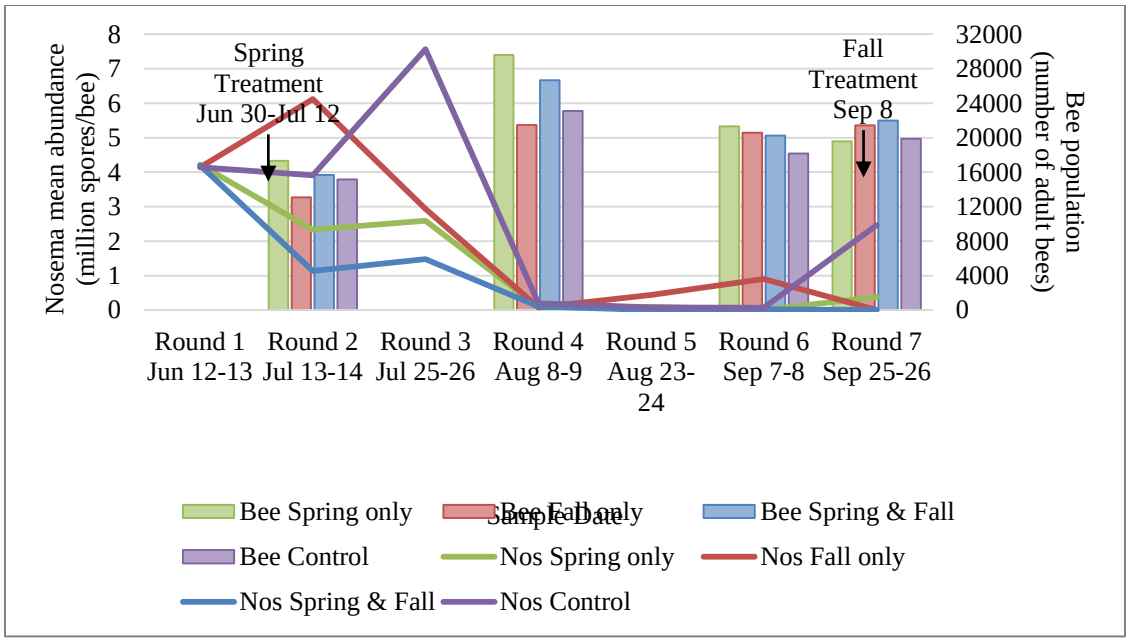

Figure 7: Change in *Nosema* mean abundance and bee population over time for all treatments in Northern apiaries. For bee population, an average of n=14 for fall only, n=15 for spring only and spring and fall, and n=16 for control.

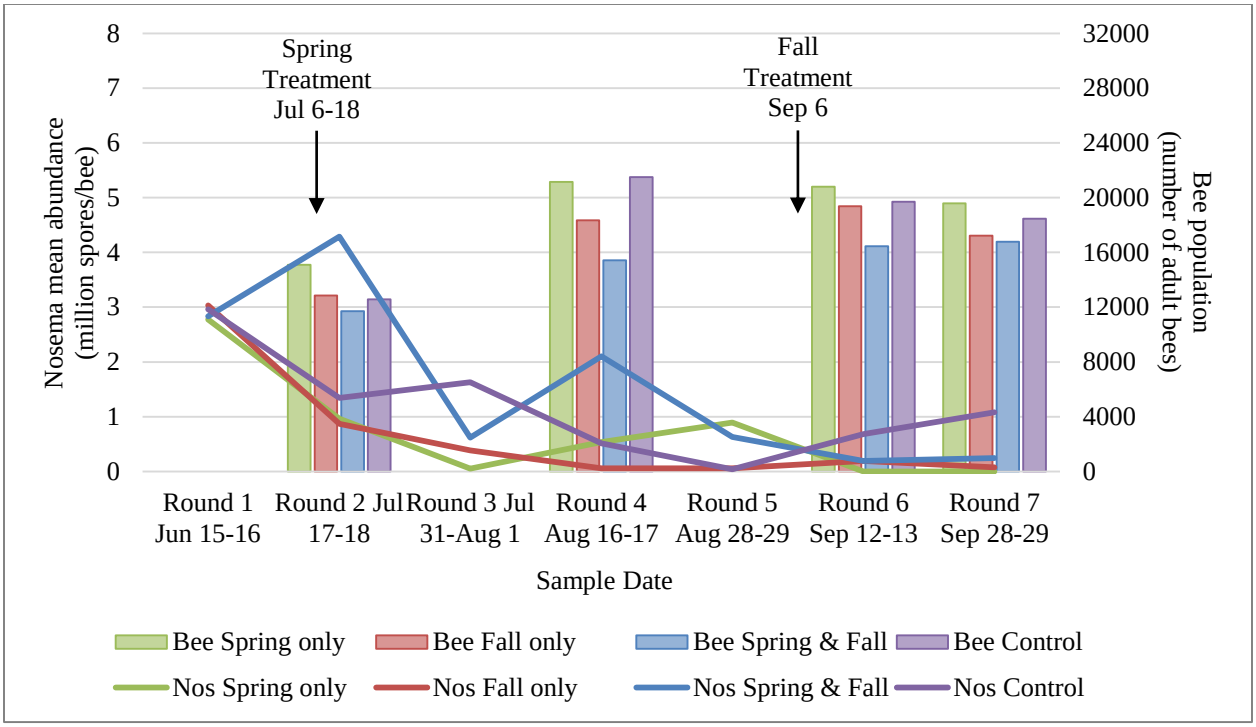

Figure 8: Change in *Nosema* mean abundance and bee population over time for all treatments in Southern apiaries. For bee population, an average of n=12 for control, n=13 for spring and fall, n=14 for fall only, and n=16 for spring only.

4. Future Research

Currently, the individual *Nosema* samples are being processed and analyzed to more accurately represent the spore levels for each colony. This will later be compared to the composite Nosema samples to determine if there is a correlation. Brood photos are being analyzed as well. Sampling will resume at the beginning of the next season. In spring 2018, colony mortality and viability will be evaluated and all surviving colonies will be sampled. The predominant species will also be determined by qPCR.

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Evaluating Potential Antibiotics for Control of *Nosema spp.* in Honey Bees (*Apis mellifera*)

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Abstract:

Nosema ceranae and *Nosema apis* cause high losses of honey bee (*Apis mellifera)* colonies in some climates. In Canada, Fumagillin (Fumagillin-B, Medivet, AB, Canada) is the only registered medication to control both species of Nosema. In our study, the effectiveness of Amprolium hydrochloride, Artemisinin, Genistein, Mebendazole, Metronidazole and Quinine on Nosema spore reproduction was evaluated using a bioassay cage study. Fumagillin was used as a reference control group for the study, as it has been a previously proven treatment method. Although the abundance and intensity of Nosema spores in live bees at the end of experiment was higher in Artemisinin and Mebendazole treatment groups, their overall bee mortality was lower compared to Fumagillin treatment groups. Collectively, Artemisinin and Mebendazole show potential to inhibit Nosema spore reproduction in honey bees, however, more research is required to support these results.

Introduction:

When it comes to honey bees (*Apis mellifera* L.), there are many diseases and pests that are able to utilize them as a host. In particular, two species of fungal pathogens (*Nosema ceranae* Fries; *Nosema apis* Zander) have been considered emerging pests of honey bees (Higes et al. 2010). High colony infection with *Nosema spp.* often prevents bee population build up in the spring, shortens adult honey bee longevity and, consequently, reduces colony survival. Fumagillin (Fumagillin-B, Medivet, AB, Canada), derived from *Aspergillus fumigatus,* is the only effective medication in the market for Nosema control since the 1950s. Although, the use of Fumagillin in honey bee colonies is banned in Europe, it is the sole medication to prevent Nosema disease in Canada. Huang et al. (2013) indicated that Fumagillin altered the structure and

metabolic proteins in honey bee midgut tissues and it may be ineffective to treat *N. ceranae*-infected colonies. This has a huge impact on Canadian beekeepers as *N. ceranae* continues to show as the dominant and most virulent species (Emsen et al. 2015). In a few investigations, synthetic antibiotics for Nosema have been tested. Gisder and Genersch (2015) found that Metronidazole and Tinidazole, in comparison with other tested medications (Quinine, Ornidazole, Albendazole, Clioquinol and Paromomycin), were able to inhibit *N. ceranae* proliferation using cell culture and cell line methods. Additionally, a study in Alberta, Canada, showed a promising efficacy of Thymol and Enicolazole for treating Nosema-infected honey bees (Heever et al. 2015). Since Nosema resistant to Fumagillin have been reported in other insects (Idris et al. 2001), resistance development in *N. apis* and *N. ceranae* can be expected. When this happens, Canadian beekeepers will suffer major losses every winter. In light of this identified gap, our trials focused on identifying alternative compounds, already on the market, for the control of *Nosema spp.* in honey bee colonies. With other alternatives in circulation, beekeepers would be able to mitigate the development of resistance of *Nosema spp.* to antibiotics, and still provide treatment to diseased colonies. Even as current research in this field has come out with some promising compounds as alternatives to Fumagillin, none have moved forward to testing on live bee colonies. Bioassays for compounds used in honey bee colonies are imperative to determine effective treatments against the pathogen, which are also safe for the host. This will ensure that beekeepers have options to manage honey bee colonies responsibly and continue to keep healthy bees.

In this project, the potential of antiprotozoal drugs; Artemisinin, Mebendazole, Metronidazole, Quinine, Genistein and Amprolium to treat *Nosema spp.* and determine their toxicity to worker bees were evaluated. Samples are being analyzed for the tested drugs. However, the results of Artemisinin and Mebendazole are reported in this report.

Objective:

The objectives of this project were to provide alternative chemicals to control *Nosema* spp, to enhance the management of Nosema developing resistance to currently used Fumagillin. Thus, beekeepers are ultimately able to keep healthy bees and reduce honey bee losses. Once the efficacy of products as well as honey bee safety data, and honey residues are developed, products can be registered with the Health Canada for use by beekeepers.

Materials and methods:

Isolation and purification of Nosema spores:

Nosema spores used for inoculation were obtained from 400–500 worker bees from highly Nosema-infected colonies on site (Crop Diversification Centre North, Edmonton, AB, Canada). Once collected, the bees were placed into smaller wooden cages (8.5x11.5x15 cm) and incubated at 34±2 ºC and 60±5% Relative Humidity (RH) for 10 days (Bahreini and Currie 2015). After 10 days, the diseased bees were placed in a freezer (-20 ºC) until 100% mortality. To obtain high concentrations of Nosema spores, the ventriculi were removed from the dead honey bee abdomens and homogenized in water with a mortar and pestle (1 mL of water per ventriculus). The concentration of Nosema spores was calculated using the hemocytometer method (Cantwell 1970). The viability of extracted spores was quantified using a staining procedure adapted from McGowan et al. (2016) using PI (Propidium Iodide), DAPI (4,6-Diamidino-2phenylindole) and fluorescent microscopy.

Bioassay procedure:

Newly-emerged honey bee collection for bioassay experiment:

In order to collect relatively disease-free worker bees, frames of capped brood about to emerge were removed from healthy, Fumagillin-free colonies and individually confined in wooden brood emergence cages (50x26x7 cm) (Fig. 1). Each emergence cage had screen on both sides for ventilation and was kept in an incubator at 34±2 °C with 60±5% RH. A sample of newly emerged bees was collected and assessed to determine the pre-experiment Nosema infection levels for each trial group. All processed samples were stored in the fridge (2-4 ºC).

Treatment cage design and set up:

Plexiglass cages (11 x12 x15 cm) were designed specifically for this bioassay experiment (Fig. 2). Inside the cages, a piece of plastic foundation was attached to the roof to allow the bees to cluster. The bottom of the cages had two removable screens to ensure removal of dead bees for Nosema spore analysis did not release the remaining live bees. The top of the cages were designed to hold four 15 mL centrifuge feeder tubes (VWR International and Fisher Scientific). A group of 24-h old worker bees (Average 97±14) were collected from brood emergence cages and placed in each Plexiglass bioassay cage before treatments were applied.

Treatment procedure

Fumagillin was used as a reference control against six candidate compounds, and 3 control groups . The candidate compounds included: Amprolium hydrochloride, Artemisinin, Genistein, Mebendazole, Metronidazole, and Quinine (Sigma-Aldrich, Canada). Three controls were used in this experiment. First control was the positive death control using Dimethoate PESTANAL® (Sigma-Aldrich, Canada) in feeding

bees in this group. The second control was the positive Nosema control that included a Nosema inoculation. The third control was the negative control group that was not inoculated or treated. Serial-dilutions for each candidate compound as well as Fumagillin were prepared using a 50% sugar solution in ddH_2O (w/w) to provide the following concentrations: 0.00001%, 0.0001%, 0.001%, 0.01% and 0.1%. Dimethoate was used to create 100% mortality at a concentration of 0.033 mg/cage () (Gough et al. 1994). As there was a limited number of specially designed bioassay cages, candidate compounds were split into 3 trials (July 5, July 27, and August 22, 2017); each trial lasting 20 days.

At the beginning of the experiment (day zero), two feeder tubes were placed on the top of each cage after a starvation period of 2 hours; one with water and the other was dependent on treatment group. Negative control colonies had a 50% sugar solution only, positive control and candidate chemical group had a 15 ml of 50% sugar solution containing Nosema spores (approximately 50,000 spores/mL) to inoculate the treatment groups. On day one of the experiment, experimental cages received feeder tubes containing serially-diluted chemical treatments in sugar solution. Death Positive control cages were fed diluted Dimethoate in sugar solution. Negative control groups continued to receive only sugar solution. All cages were then provisioned *ad libitum* with 50% sugar solution containing selected concentrations of each candidate chemical, Fumagillin, or Dimethoate for 20 days and refreshed as needed. Positive and negative control groups were provisioned *ad libitum* with sugar solution only for the duration of the experiment. All bioassay cages were randomly placed in a controlled-environment incubator at 33±1 °C with 70±2% RH in the dark.

Data collection and statistical analyses:

The duration of the bioassay experiment was 20 days post-inoculation. Temperature and relative humidity were recorded using HOBO data loggers (Onset Computer Corporation, MA, USA). Daily consumption of sugar solution was also quantified and recorded. Daily throughout the 20 days, dead bees were collected, counted, and processed in labelled sample tubes. At days 5, 10, and 15 (dependent on trial), five live bees were randomly selected and removed from each cage and processed in sample tubes. Both live and dead bees, with the exception of bees collected on day 20, were processed as composite samples using tissue grinders (1mL water per whole bee abdomen). On day 20, the remaining live bees were collected and individually processed to determine *Nosema* spore intensity (millions spores per infected-bee), abundance (millions spores per bee in the sample), and infection prevalence (percentage of bees infested in the sample) (Bush et al. 1997). Individual and composite samples were prepared by removing the whole abdomen and grinding the samples in water (1 mL of water per abdomen) (Fig. 3). The Nosema spore intensities and abundance were calculated using the hemocytometer method (Cantwell 1970).

he bioassay experimental design was a split plot treatment arrangement in a randomized complete block design with ten treatments (six candidate compounds, one reference control, one death control, one positive control and one negative control), five concentrations for candidate compounds and Fumagillin treatments, and three replicates for each concentration. For this experiment, the candidate compound and Fumagillin treatments are the main plots (n=7) and concentrations are the sub-plots.

The effect of treatments on bee longevity was analyzed using the Kaplan-Meier survivorship analysis (PROC LIFETEST, SAS, 2011). Prior to analyses, variables of bee mortality abundance and intensity of Nosema spores were log-transformed. The interaction of treatments and concentrations on daily bee mortality were analyzed by ANOVA using a repeated measure analysis of variance; treatments and concentration as main effects and day as repeated measure (PROC MIXED, SAS, 2011). Where significant

interactions were observed, differences among treatment means were compared using Bonferroni-corrected contrast (SAS, 2011).

Results:

Nosema infection prevalence was similar in all inoculated treatments and all were significantly higher than the negative control (F= 31.6 df= 4, 2274; P < 0.0001) (Fig. 4). Positive control groups had a significantly higher intensity (F= 21.37; df= 4, 1703; P< 0.0001) and abundance (F= 31.06; df= 4, 2274; P< 0.0001) of Nosema spores than negative controls; followed by Mebendazole, Artemisinin, and Fumagillin (Fig. 5 and 6). Abundance of Nosema spores in dead bees significantly increased after day 9 in all treatments except the negative control (F= 3.01; df= 5, 46; P=0.0197) (Fig. 7 and 8). Dimethoate caused 100% percent mortality in tested bees during the first 7 days post-treatment. Fumagillin, Artemisinin and Mebendazole had similar spore abundance in dead bees (F= 3.01; df= 5, 46; P= 0.0197) (Fig. 10) as well as the live bees collected at days 5, 10 and 15 post-inoculation (F= 5.21; df= 5, 48; P=0.0007) (Fig. 11). Additionally, lower bee mortality was evaluated for Artemisinin and Mebendazole in comparison to Fumagillin treatments (F= 26.94; df= 5, 49; P< 0.0001) (Fig. 9). The Kaplan-Meier survival analysis showed no significant differences in worker bee mortality when bees were exposed to Mebendazole, Artemisinin or left untreated. More than 70% of the bees were still alive even on day 20 post-inoculation in Artemisinin, Mebendazole and negative control whereas more than 70% the bees were dead in Fumagillin and positive control treatments (Long-Rank : X^2= 2980.1176 ; df= 5; P < 0.0001) (Fig. 12).

Discussion:

Dimethoate treatments died within the first 7 days post treatment indicating that honey bees do have sensitivities to low concentrations of toxic chemicals in sugar feed (Gough et al. 1994). Treatments inoculated with *Nosema* spores had also significantly higher spores counts than the uninoculated control. Treatments with higher concentrations of Fumagillin did reduce the abundance and intensity of spores in samples, showing it is still an effective treatment at this time as long as the correct dosage is used according to the label recommendation. In comparison to Fumagillin, *Nosema* spp. abundance, and intensity increased in Artemisinin and Mebendazole were higher by (57-96%). However, these tested chemicals showed a significantly lower mortality to adult bees. This suggests that these compounds are safe for honey bees if applied as agents to inhibit Nosema spore production. In addition, the rate of infected bees (prevalence) was not significantly different among tested compounds. Spore counts in dead and live bees that were collected throughout the trials indicated that Nosema spores increased in inoculated-bees during 10 days post-inoculation for all compounds.

Our results show the toxicity of higher doses of Fumagillin for bees in comparison with lower concentrations. This indicates that if beekeepers do not follow the label recommended dose, there is potential to kill adult bees. Lower concentrations of Fumagillin, as well as lower doses of Mebendazole and Artemisinin showed low toxicity to adult bees. The survival curve indicated that the survival of adult bees treated with low doses of Mebendazole and Artemisinin were comparable to bees that were not treated at all. The comparison of these two chemicals to Fumagillin showed that more bees survived in the candidate tested compound low dose treatments than in low dose Fumagillin treatments.

Overall, Nosema prevalence was similar among tested compounds, however, abundance and intensity increased significantly in live bees at the end of trials for Mebendazole and Artemisinin in comparison to Fumagillin. at .1 Despite t these, results indicate potential to be used as an alternative chemical medication to reduce *Nosema* spores. Higher concentrations of compound must be tested again to determine efficacy and toxicity to bees to find proper dose to recommend for treatment. The samples

collected from testing four more candidate chemicals (Metronidazole, Quinine, Amprolium, and Genestein) are currently being analyzed. It will be reported at a later time. The candidate chemicals that showed high efficacy against Nosema under laboratory conditions will be subjected for further testing in the field using bee colonies. Successful field trials with promising candidate chemicals would be continued to collect all necessary data on efficacy, bee safety and residues in honey to use for registration with Health Canada. This is a priority research because the longer we wait to replace antibiotics on the market with alternatives, the higher the probability that pathogens will become resistant.

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Fig. 1: Brood emergence cages.

Fig. 2: Plexiglass bioassay cage with four 15mL feeding tubes.

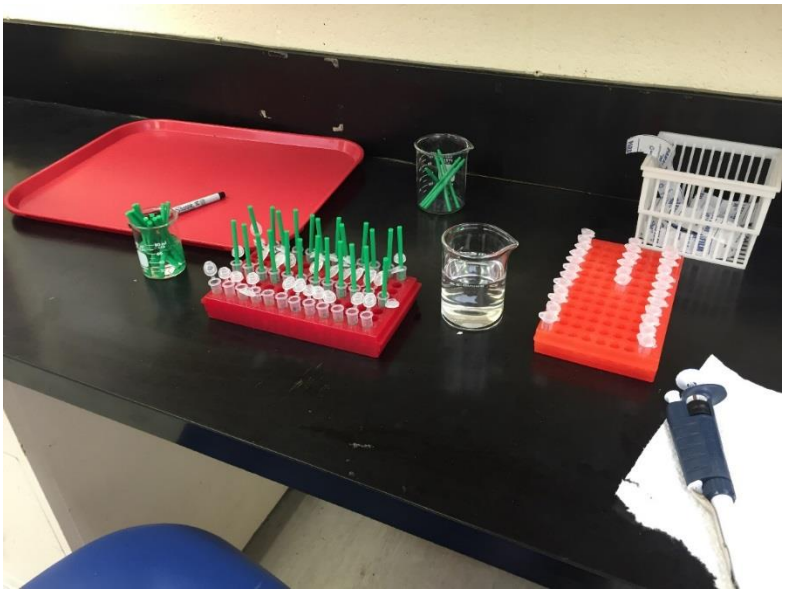

Fig. 3: Individual honey bees were crushed in a 1.7 mL centrifuge tube containing 1 mL water to calculate Nosema spore (millions of spores per bee).

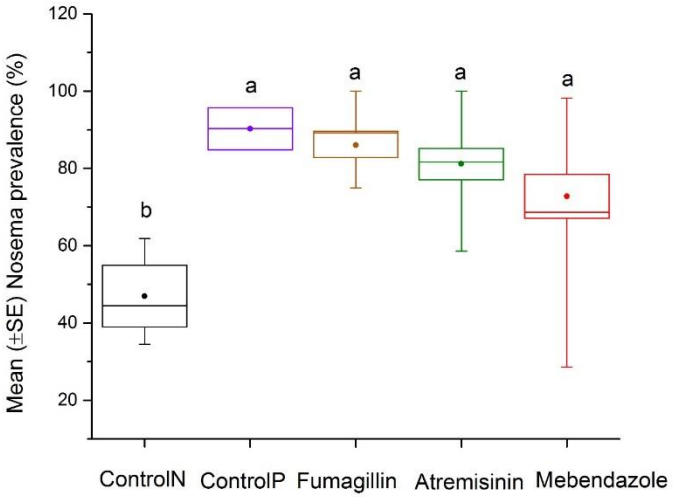

Fig 4: Mean (±SE) Nosema prevalence (%) in live bees that were collected at day 20 post-inoculation in different treatments. The boxplots indicate the standard error (length of box), mean (solid dot), median (horizontal line inside box), 5th and 95th percentiles (lower and upper vertical lines). Means of treatments with the same letter are not significantly different.

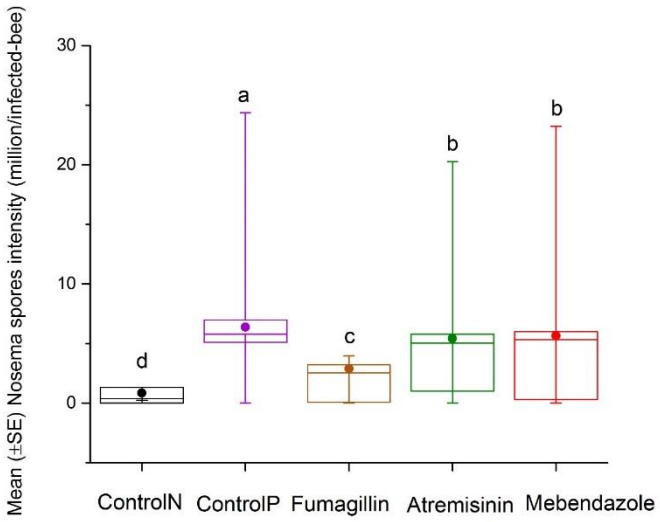

Fig 5: Mean (±SE) Nosema spores intensity (million per infected-bee) in live bees that were collected at day 20 post-inoculation in different treatments. The boxplots indicate the standard error (length of box), mean (solid dot), median (horizontal line inside box), 5th and 95th percentiles (lower and upper vertical lines). Means of treatments with the same letter are not significantly different.

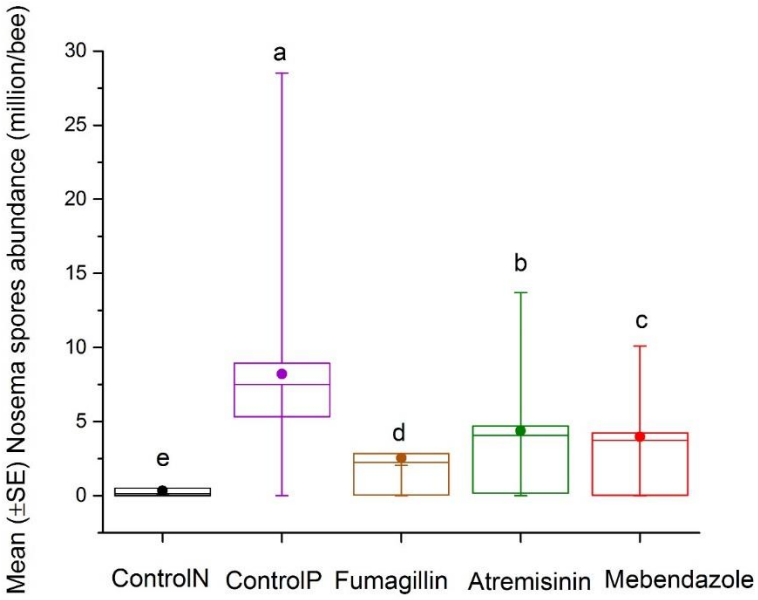

Fig 6: Mean (±SE) Nosema spores abundance (million per bee) in live bees that were collected at day 20 post-inoculation in different treatments. The boxplots indicate the standard error (length of box), mean (solid dot), median (horizontal line inside box), 5th and 95th percentiles (lower and upper vertical lines). Means of treatments with the same letter are not significantly different.

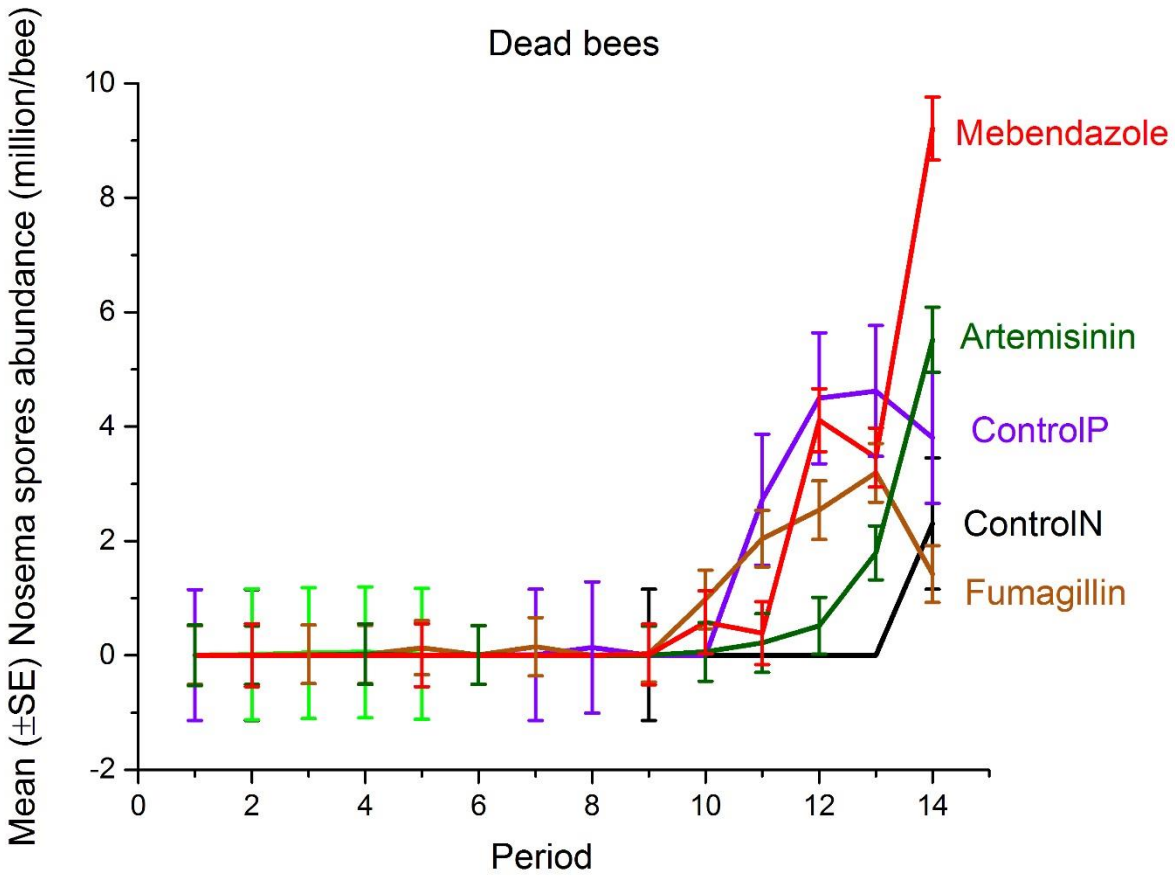

Fig 7: Mean (±SE) Nosema spore abundance (million per bee) in dead bees across treatments.

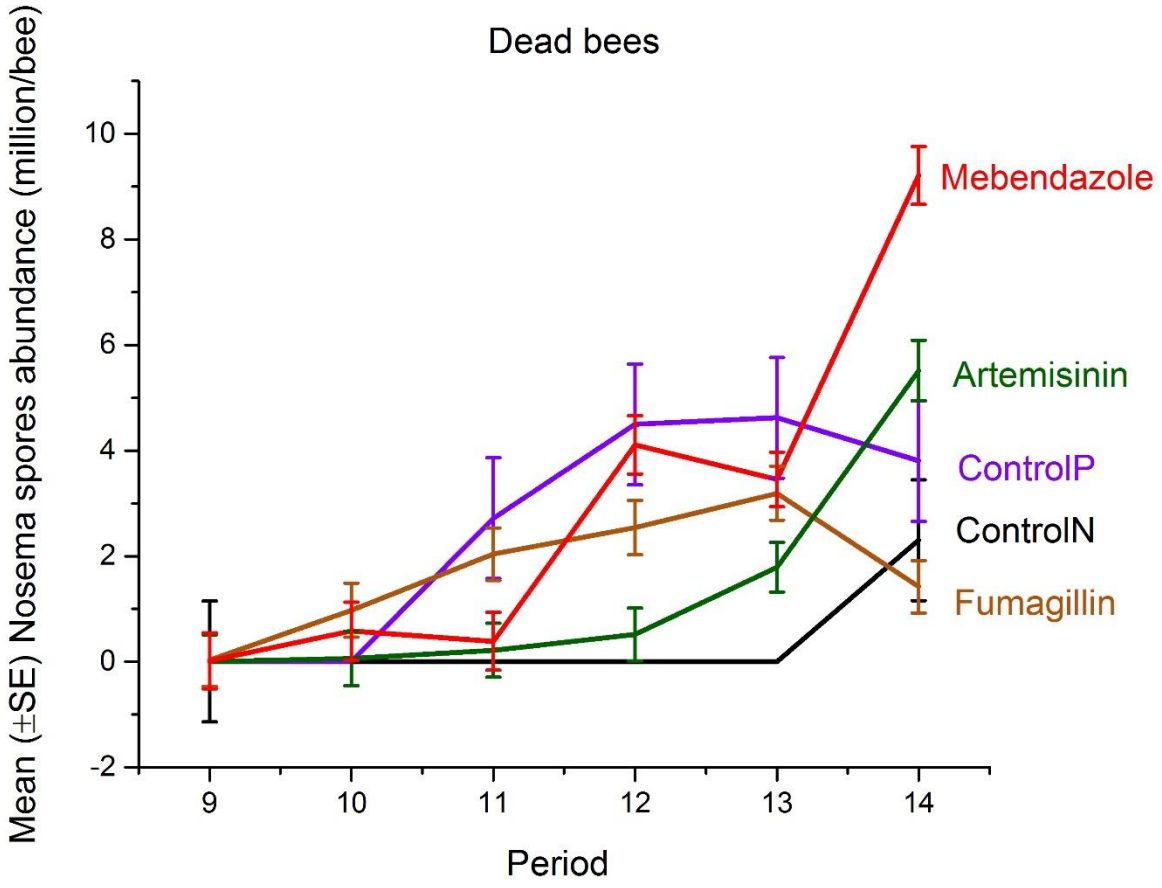

Fig 8: Mean (±SE) Nosema spores abundance (million per bee) in dead bees that were collected between days 9 and 20 in different treatments.

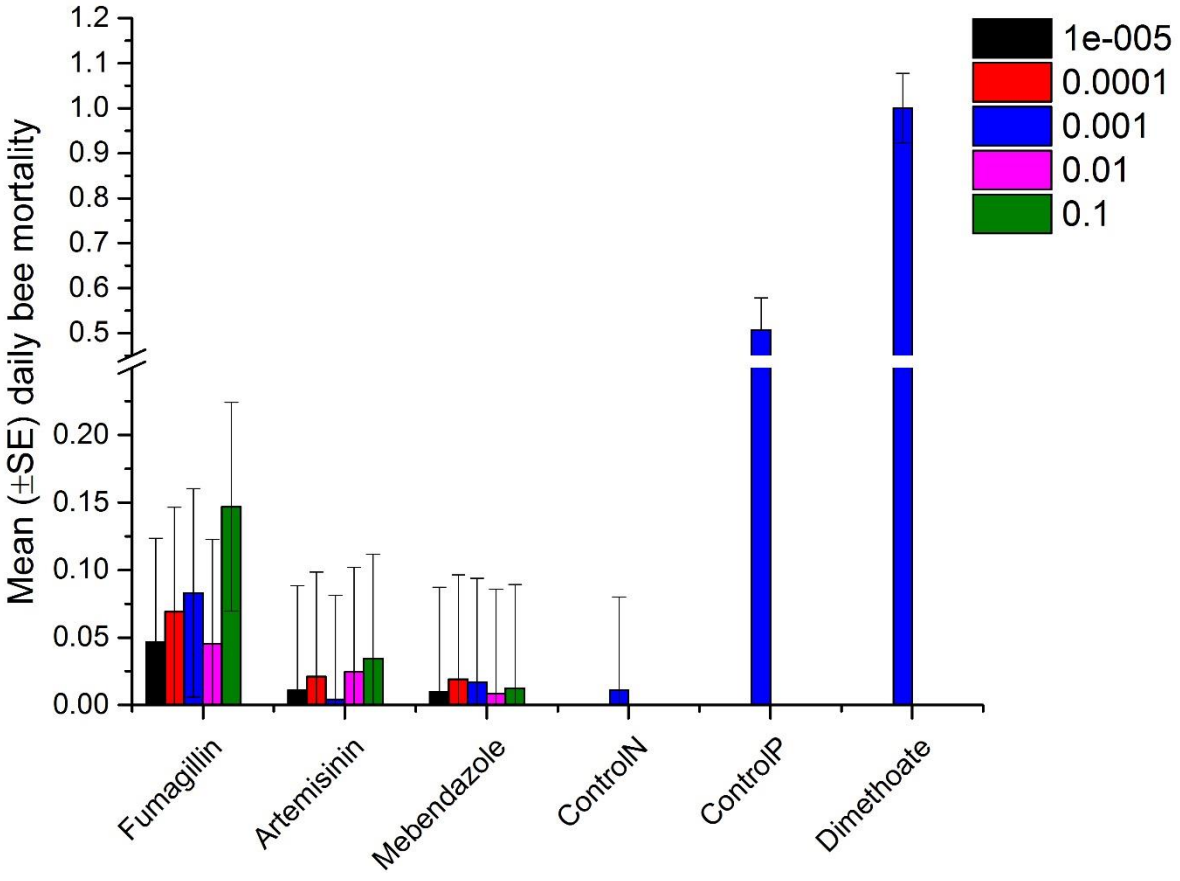

Fig. 9: Cumulative mean (±SE) daily mortality of worker bees in different treatments collected post-inoculation.

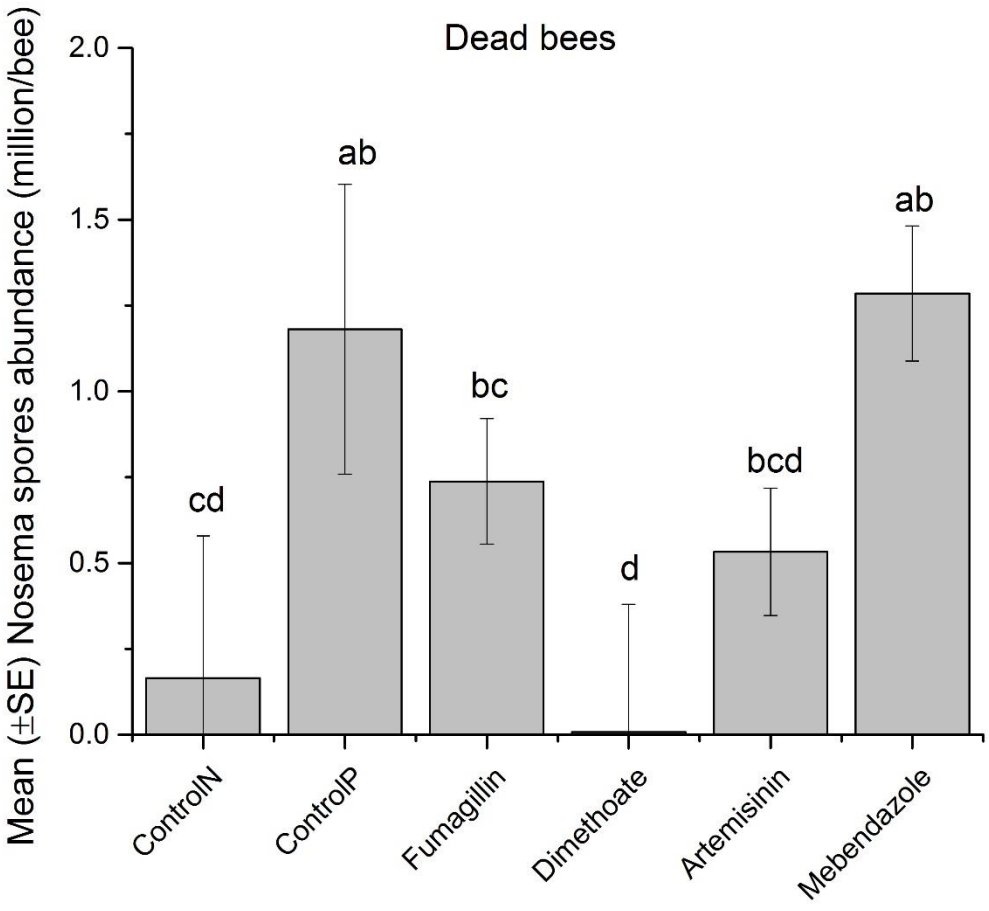

Fig 10: Cumulative mean (±SE) Nosema spore abundance (million per bee) in dead bees across treatments. Means of treatments with the same letter are not significantly different.

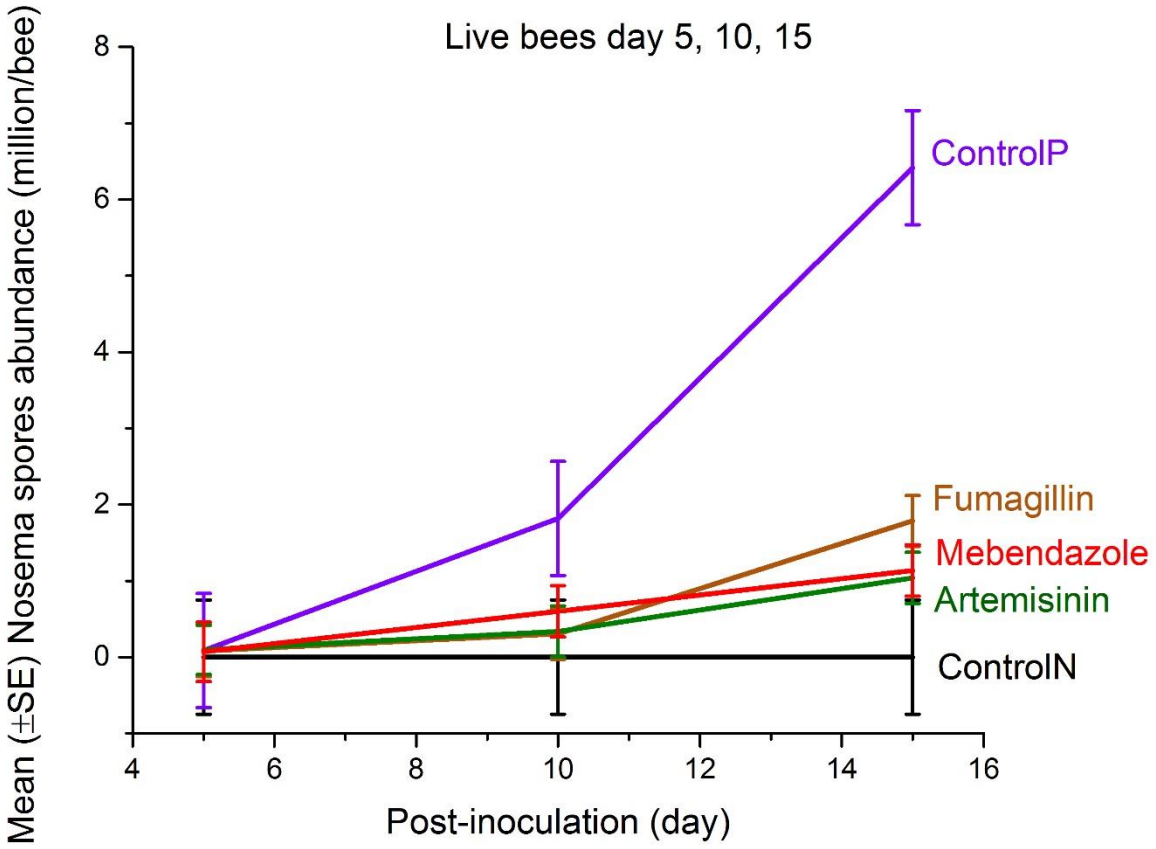

Fig 11: Mean (±SE) Nosema spore abundance (million per bee) in live bees collected at days 5, 10 and 15 post-inoculation.

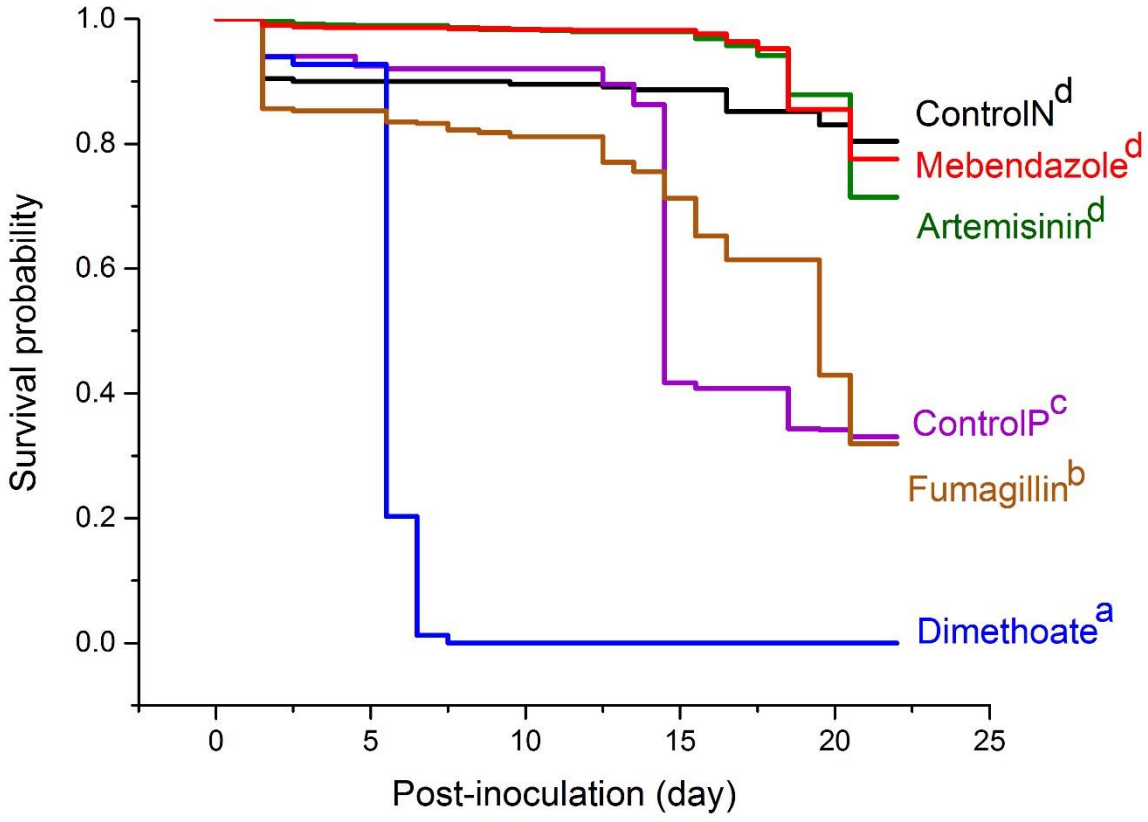

Fig 12: Survival plot of worker bees across experimental treatment groups. Treatments followed by the same letter are not significantly different.

Mater Bee Health App Pests Information

Name:
American Foulbrood

Description:
American Foulbrood (AFB) is a disease caused by a spore-forming bacteria. AFB spores can live for decades in infected equipment. If left untreated, AFB will cause widespread colony death. Prompt identification and action must be taken to avoid the spread of AFB.

Type:
Brood

Symptoms:
AFB is caused by the bacteria *Paenibacillus larvae larvae.*

- Spotty brood pattern.
- Dark, sunken, perforated, greasy-looking cappings.
- Larvae turn from dull white, to yellowish, to coffee-coloured.
- Decomposing larva can be drawn out in a ropy thread at least 1.5 cm long.
- Fine, threadlike pupal tongue reaching across the cell.
- Dark scale lies flat on lower side of cell and adheres to the cell wall, making it hard to remove.
- Strong fishy odour.

Monitoring Information:

- Check at least three frames of brood per colony every 10 days during active season for the above symptoms.
- If a suspected infected cell is found, use a toothpick, matchstick or a hard piece of grass to test how dead larva can be drawn out of cells (rope more than 1.5 cm = AFB).
- For confirmation, Alberta registered beekeepers can mail the sample(s) to Alberta Agriculture and Forestry – Apiculture Laboratory for testing.
- Take all biosecurity precautions to avoid spreading AFB spores into other bee colonies**.**

Management Treatment Options:

To Prevent AFB infections:

- Inspect brood combs for AFB throughout the season so any infection can be diagnosed and treated as early as possible.
- Use queens from genetically resistant stock.
- Replace 3-4 old brood combs per year with fresh comb or foundation.
- Do not transfer combs from hive to hive and do not divide or unite colonies unless they are free from AFB.
- Be mindful of captured swarms. They may be contaminated with AFB spores. Place collected swarms into a hive in a separate apiary until the colony's health is certain and no infection is found.
- Regularly sterilize beekeeping equipment (hive tools, gloves, bee brush) and wash hands with soap and water.

- If the beekeeping suit and veil are suspected to be contaminated, please wash before moving to another bee yard.
- Irradiate dead outs and hive boxes. Never reuse infected combs unless they are sterilized using irradiation.
- If you are saving infected empty bee boxes, bottoms boards and lids, you must disinfect this equipment before re-use by scorching, high pressure water washing, or Virkon (available from animal supply stores or veterinary drug stores).
- Any infected combs should be stored in a bee-proof facility until they are burnt or irradiated. This prevents bees from robbing and spreading AFB to other bee colonies in your operation or other operations near you.

Chemical Treatment Options:

Infected colonies require immediate and long term treatments. A beekeeper must determine the level of AFB infection. Based on the infection level, one of the following registered antibiotics by Veterinary Drug Directorate, Health Canada can be used. Please consult with your provincial apiculturist or a veterinarian in your region for advice.

Oxytetracycline:

- Oxy-Tet-25-S *(DIN 0223111)* (Check the label)
- Oxy Sol – 62.5 (*DIN 00560189)* (Check the label)

- If colonies are heavily infected and weak, immediately burn hives with bees. Antibiotics will not help at this point.
- If a medium infection with medium to large size bee colonies, use the shook swarm method. This method includes shaking only bees onto new equipment with foundation to get rid of as many AFB spores as possible. Repeat this step one more time if needed. Oxytetracycline can be used to treat bees in new equipment to stop reinfection. Burn all infected equipment.
- In the case of a light infection with medium to large colonies, remove and burn infected frames.
- Note: Treat bees with oxytetracycline following label instructions and use oxytetracycline in powdered sugar mix, not in sugar syrup; oxytetracycline decays quickly in sugar syrup.

Tylosin:

- If symptoms persist after using oxytetracycline, send samples to test for oxytetracycline resistance.
- Use Tylosin according to the label for treatment of oxytetracycline-resistant strains of AFB.
- Note: If a beekeeper plans to export honey to Japan, Tylosin cannot be used.

Name:

Bears

Description:

Black bears tend to cause more problems in bee yards than grizzly bears. Bears will topple hives and wreck equipment in order to eat honey and bee brood. They can destroy a bee yard very quickly.

Type:

Hive

Symptoms:

- Knocked over hives with broken hive equipment strewn about.
- Full hive boxes drug to the nearest bush with the contents eaten.

Monitoring Information:

- Check apiaries for any suspected bear damage.

Management Treatment Options:

- Place bear fencing around bee yards in known bear territory. Bear fences are most effective before a bear develops a taste for honey and bees; fencing a bee yard after a bear has caused problems may not deter the bear from returning.
- Contact your wildlife specialist to report incidents and request information on legal action for bear control.

Chemical Treatment Options:

- There is no registered chemical control for bears attacking apiaries.

Name:

Bee Louse

Description:

The bee louse is a wingless fly that can live on queens and worker bees. The bee louse feeds on wax and pollen and can cause minor damage. They can be mistaken for varroa mites.

Type:

Adult

Symptoms:

- The bee louse, *Braula coeca*, is a 1.2-1.5 mm reddish brown wingless with a rounded appearance with 6 legs, while adult varroa mite has eight legs with a compressed oval body.
- It may be seen on the bee's body but primarily on queens.
- Larva tunnel into capped honey.
- It can be found with imported bee packages but tends not to persist in Alberta.

Monitoring Information:

- Check adult bees, primarily queens, for bee louse. They could be located anywhere on the bee's body.
- Check capped honey for the bee louse larva tunnels.
- Check imported bee packages for bee louse on bees.

Management Treatment Options:

- Management measures are not needed.

Chemical Treatment Options:

- Control measures are not needed.

Name:

Black Queen Cell Virus

Description:

Black queen cell virus (BQCV) is a viral disease. It infects developing queen larvae and causes them to turn black and die. When it is present in colonies used for queen cell building, attempts at queen rearing may fail. It can be present in adult bees without visible symptoms. It may possibly be associated with nosema and varroa mites.

Type:

Brood

Symptoms:

- Dead queen pupae turn yellow, darken to brown, and then black.
- Exterior of queen cell looks darker once queen larva has turned black.

Monitoring Information:

- Check developing queen cells in queen cell builder colonies for the above symptoms.
- Monitoring, detection, and quantification of BQCV can be done by taking adult bee samples and sending them to labs for molecular assays.

Management Treatment Options:

- Sanitize grafting tools in ethanol or with fire.
- Use new queen cell cups only; do not reuse queen cups.
- Use strong colonies for all stages of queen rearing.

Chemical Treatment Options:

- There is no specific registered chemical treatment for BQCV.
- Treating for nosema and varroa mites in queen cell builders will help reduce the impacts of BQCV on developing queens.

Name:

Chalkbrood

Description:

Chalkbrood is caused by a fungus that kills larvae and turns them into white or blackish mummies. It is more prevalent in bee colonies in spring and early summer. It is associated with stress caused by inclement weather. Bees will often remove mummies from cells and carry them to the hive entrance.

Type:

Brood

Symptoms:

Chalkbrood is caused by the fungus *Ascophaera apis*.

- White, grey or black mummies in brood cells, on bottom boards and around the hive entrance.
- Punctured brood cappings.
- Spotty brood pattern with mummies in sealed and unsealed brood cells.
- Mummies do not adhere to cells.

Monitoring Information:

- Check at least three frames of brood per colony every 10 days during the active season for the above symptoms.

Management Treatment Options:

- Requeen with a new queen or preferred hygienic queen stocks.
- Increase hive ventilation.
- Place hives in a warmer area or face them south
- Replace heavily infected comb with fresh comb or foundation.
- Keep strong bee colonies.

.Chemical Treatment Options:

There is no registered chemical treatment for chalkbrood.

Name:

Chilled Brood

Description:

Developing brood is kept warm (34-35°C) by adult bees in the bee cluster. If there are not enough adult bees to keep the brood warm, brood will become chilled and die. This commonly occurs in early spring, when the queen is expanding the brood nest area but there is not a large population of adult bees yet. If the weather turns colder and the adult bees contract into a small cluster, brood out of the cluster area will become chilled. Chilled brood may also occur if the adult bee population suddenly declines for some reason, such as pesticide poisoning.

Type:

Brood

Symptoms:

- Dead larvae becoming dark and flattened in cells.
- Dead larvae do not rope.
- Dried scale easily removed from cell.
- Dead larvae and pupae often found on outer areas of brood frames and outer frames of brood chambers.

Monitoring Information

- Check brood frames for dead brood in outer areas of the brood frames.

Management Treatment Options:

- Use entrance reducers in spring.
- Reduce number of hive boxes to keep brood warm at all times
- Keep strong bee colonies.
- Keep winter wraps on until daytime temperatures are consistently above 15°C.

Chemical Treatment Options

There is no chemical control for treatment of chilled brood.

Name:

Drone Laying Queen

Description:

A queen can lay two types of eggs: fertilized eggs, which become workers, or unfertilized eggs, which become drones. If a queen runs out of sperm in her spermatheca, she can only lay unfertilized eggs. A queen that lays only unfertilized eggs is called a drone laying queen.

Type:

Hive

Symptoms:

- Protruding capped brood in worker cells caused by drone brood developing in worker cells.
- Spotty brood pattern.
- Single or multiple eggs on cell bottom.

Monitoring Information

- Check for queen and normal worker brood pattern in brood combs.
- Check for scattered capped drone brood in worker cells.

Management Treatment Options:

- If the colony is strong, remove the drone laying queen. Introduce a new queen along with one or two young brood combs without the bees (only if disease free).
- If the colony is smaller, remove the queen and combine the colony with another colony if there are no apparent diseases.
- Shake a weak drone layer colony out in the apiary and remove the hive.

Chemical Treatment Options:

- There is no registered chemical control for treatment of drone laying queens.

Name:

Egg Laying Workers

Description:

When a bee colony has been queenless for some time, worker bees may begin laying eggs. These bees are called egg laying workers. Their eggs are not fertilized so they can only develop into drones.

Type:

Hive

Symptoms:

- Scattered capped drone brood in worker cells.
- Colony is queenless.
- Multiple eggs in one cell.
- Eggs on sides of cell instead of the bottom of the cell because the worker's abdomen is too short to reach the bottom of the cell.

Monitoring Information:

- Check for the presence of a queen and normal egg laying and worker brood pattern in brood combs.
- If a colony becomes queenless, check for multiple eggs in worker cells and on side walls of worker cells.
- Check for scattered capped drone brood in worker cells.

Management Treatment Options:

- To prevent egg laying workers from developing, give queenless colonies a frame of eggs and young larvae from another colony (if disease free) when it first becomes queenless.
- Re-queening colonies that have egg laying workers generally does not work.
- If there is a large number of egg laying workers in a colony, shake all the bees out of the colony in the apiary and remove the hive.

Chemical Treatment Options:

- There is no registered chemical treatment for egg laying workers.

Name:

European Foulbrood

Description:

European Foulbrood (EFB) is a bacterial disease. It can be a sign the bees are stressed by inadequate nutrition or non-ideal weather conditions. When conditions improve and colonies strengthen, symptoms of light EFB infections may disappear. However, in some cases it can be quite severe.

Type:

Brood

Symptoms:

EFB is caused by *Melissococcus plutonius*.

- Unsealed brood with curled whitish-yellow to brown larva, often with visible tracheal tubes.
- In severe cases there will be partially capped brood or discoloured, sunken or punctured cappings with curled larvae in cells.
- Decomposing larva may be drawn out in a ropey thread less than 1.5 cm.
- Rubbery larval scale retains the curled shape on bottom of cell and is easy to remove.
- May have a sour smell.

Monitoring Information:

- Check at least three frames of brood per colony every 10 days during the active season for the above symptoms.
- If a suspected infected cell is found, use a toothpick, matchstick or a hard piece of grass to test how dead larva can be drawn out of cells (Rope less than 1.5 cm = EFB).
- For confirmation, take samples and mail to Alberta Agriculture and Forestry for testing.
- Take all biosecurity precautions to avoid spreading EFB spores into other bee colonies.

Management Treatment Options:

To Prevent EFB infections:

- Inspect brood combs for EFB throughout the season so any infection can be diagnosed and treated as early as possible.
- Use queens from genetically resistant stock.
- Do not transfer combs from hive to hive and do not divide or unite colonies unless they are free from EFB.
- Be mindful of captured swarms. They may be contaminated with EFB spores. Place new swarms into a hive in a separate beeyard until the colony's health is certain and no infection is found.
- Regularly sterilize beekeeping equipment (hive tools, gloves, bee brush) and wash hands with soap and water.
- Replace 3-4 old brood combs per year with fresh comb or foundation.
- Irradiate dead outs, infected combs, and hive boxes. Never reuse infected combs unless they have been irradiated.
- If you are saving infected empty bee boxes, bottoms boards and lids, disinfect before re-use by scorching, high pressure water washing, or Virkon (available from animal supply stores or veterinary drug stores).

Chemical Treatment Options:

Based on the infection level, one of the following registered antibiotics by Veterinary Drug Directorate, Health Canada can be used. Please consult with your provincial apiculturist or a veterinarian in your region for advice.

Oxytetracycline:

- Oxy-Tet-25-S *(DIN 0223111)* (Check the label)
- Oxy Sol – 62.5 (*DIN 00560189)* (Check the label)

Follow label instructions: It is highly recommended to use Oxytetracycline in powdered sugar mix, not in sugar syrup solution due to the fast decay of Oxytetracycline in sugar solution.

Name:

Nosema

Description:

Nosema is caused by fungal microsporidia. It is a serious disease in northern temperate climates. Nosema spores enter the bee's midgut and multiply. Millions of spores are then shed in the bee's feces.

Type:

Adult

Symptoms:

Nosema can be caused by either *Nosema apis* or *Nosema ceranae.* While *Nosema apis* infections are most pronounced in early spring, *Nosema ceranae* infections may be problematic at any time of the year.

Visible indicators include:

- Bee feces on brood comb and/or at hive entrance.
- Poor spring colony population build-up.
- Reduced honey production.
- Early queen supercedure.
- Colonies often die over winter.

Monitoring Information

For a definitive diagnosis collect a sample of 30- 60 older bees from honey combs or the inner cover in 70% alcohol or winter windshield washer fluid. Once collected, send to an apiculture specialist or laboratory to have a microscopic examination done.

Threshold: If the average number of spores exceeds 1 million spores/bee, colonies should be treated.

Management Treatment Options:

- Replace infected equipment.
- Irradiate infected equipment.
- Fumigate infected equipment with acetic acid.

- Ensure hives are well insulated and draft-free in winter.
- Place hives facing south or in warmer areas to allow for cleansing flights in winter.

Chemical Treatment Options:

- Fumagillin is the only registered effective antibiotic treatement for nosema. Application methods depend on season and health of colonies. Refer to product labels for instructions on specific product use.

Name:

Parasitic Mite Syndrome

Description:

Colonies infested with Varroa mites can exhibit a large array of symptoms affecting both brood and adult bees. This grouping of symptoms is referred to as Parasitic Mite Syndrome (PMS).

Type:

Brood

Symptoms:

Not all symptoms are found in a colony at the same time.

- Spotty brood pattern.
- Affected larvae can be twisted or melted in the cells.
- Affected larvae can be white, light brown or black.
- Larvae can have a watery to pasty consistency and be similar in appearance to EFB, AFB and sacbrood.
- Larvae do not rope.
- Presence of *Varroa* mites on face of combs or in both capped and uncapped cells.
- Lack of eggs and developing larvae.
- Decreased adult bee population.
- Crawling bees and bees with deformed wings at hive entrance.

Monitoring Information

- Check developing larvae and pupae for the above symptoms.
- Monitor *Varroa* mite infestations.

Management Treatment Options:

- Control *Varroa* mites.
- Requeen using queens from mite tolerant or hygienic bee stocks.
- Keep strong bee colonies.

- Disinfect combs from dead-outs using irradiation.

Chemical Treatment Options:

- There is no registered chemical treatment.

Name:

Pesticide Poisoned Honey Bees

Description:

Foraging honey bees can come into contact with agricultural pesticides and in-hive pesticides. The main exposure routes for honey bees are: direct spraying on crops while bees are foraging on flowers, contact with treated crops, unsafe exposure to pesticides applied to bee colonies, and feeding on contaminated nectar and pollen. These exposures can lead to a serious poisoning, weakened bee colonies and a sudden loss of bees.

Type:

Hive

Symptoms:

- Dead and dying honey bees in front of the hives.
- At the hive entrance and inside the hive, bees may suffer from paralysis and/or display abnormally jerky, wobbly, or rapid movements. This includes twitching and spinning on their backs.
- Increased defensiveness (a symptom of exposure to most insecticides).
- Immobile, lethargic bees unable to leave flowers.
- Regurgitation of stomach contents and tongue extension.
- The appearance of crawling bees unable to fly and moving slowly, as though they have been chilled.
- Dead brood.
- Dead newly emerged workers.
- Abnormal queen behaviour, including queenless bee colonies.

Monitoring Information:

- Measure the amount of dead adult bees outside and inside the hive.
- Check colony strength.
- Check brood for dead or removed larvae or pupae.
- Check for queen supersedure cells and if the hive is queenless.
- Collect bee, brood, honey and wax samples to test for pesticides.
- Any finding of suspected pesticide bee poisoning should be reported to the Pest Management Regulatory Agency (PMRA) and the Provincial Apiculturist for investigation.

Management Treatment Options

- Beekeeper-grower cooperation is the most effective way to reduce bee poisoning.
- Beekeepers, growers and pesticide applicators must develop working relationships and familiarize themselves with each other's management practices.
- Honey bee crop pollination contracts should include details to safeguard honey bees from poisoning.
- Beekeepers and growers should use safe pest management practices.
- If hives have pollen and honey contaminated with pesticide residues, these hives should not be reused for bees.
- Use care in controlling pests in and around beehives, apiaries and beekeeping storage facilities.
- Use insecticides labeled for the intended use and follow all label directions carefully.

Chemical Treatment Options:
There is no chemical control applicable for poisoned bees or contaminated pollen and honey.

Name:

Sacbrood

Description:

Sacbrood is a virus that kills brood. It may appear at any time during the brood-rearing season. It does not usually cause severe losses.

Type:

Brood

Symptoms:

- Cells with punctured cappings scattered throughout sealed brood.
- Larva will be upright, sac-like and stretched out in cell.
- A tough leathery membrane makes larva easy to remove from cells.
- Larvae turn from white, grey, yellow to brown and then black with the head end darker.
- Infected larvae do not adhere to cell walls.

Monitoring Information:

- Check at least three frames of brood per colony every 10 days during active season for the above symptoms.

Management Treatment Options:

- Requeen with a new queen; or preferred hygienic stock.

- Replace heavily infected comb with fresh drawn comb or foundation.
- Maintain strong, healthy colonies.

Chemical Treatment Options:
There are no registered chemical treatments.

Name:

Skunks

Description:
Skunks can be a problem in apiaries because they eat bees. They are mostly active at night. They scratch the hive's entrance and when the bees come out responding to the disturbance, the skunk will eat them. Bee colonies can be weakened.

Type:

Hive

Symptoms:

- Grass flattened in front of hive.
- Small hole dug in front of the hive entrance.
- Scratches on hive boxes and dirt on the entrance board.
- Aggressive hives.
- Skunk feces filled with bee skeletons.
- Torn winter wraps.
- Skunk tracks in snow.

Monitoring Information:

- Check apiaries for symptoms of skunk damage.

Management Treatment Options:

- Trapping. There are often multiple skunks in a bee yard. Keep trapping until no more skunks are caught.
- Contact your wildlife specialist to report incidents and ask what legal action can be taken to control skunks.
- Electric fencing or chicken wire fencing.
- Place barriers in front of hives to prevent skunks from scratching at entrances. Barriers include: carpet tack strips, wire mesh, small flowerbed wire fences, and boards with nails.

Chemical Treatment Options:
There is no registered chemical control for skunks in apiaries.

Name:

Small Hive Beetle

Description:

The small hive beetle (SHB) is an emerging pest of the honey bee in Canada. It is a member of the sap beetle family. It is an opportunistic scavenger. It can adversely affect all beekeeping activities including bee survivorship, honey production and processing and pollination. It is found in Quebec, Ontario, Manitoba and British Colombia. Control measures have been taken to control SHB and limit its establishment in Canada.

Type:

Hive

Symptoms:

- Adult, larvae and eggs of the SHB, *Aethina tumida*, can be seen in honey bee colonies.
- The population of SHB can build-up quickly, and suddenly hundreds to thousands of larvae can appear. Adults can be found on the underside of the inner cover, top bars of frames and on the bottom board.
- The SHB distributes the yeast *Kodamaea ohmeri*, which ferments honey and pollen, creating a slimy mess on the surface of combs, honey running out of the hive and a strong odor of rotting orange.
- SHB larvae will leave the colony to pupate in the soil.
- Weak colonies can be heavily damaged and bees abscond.

Monitoring Information:

- Inspect bee colonies on a warm day. The adult SHB is often found in dark, tight spaces in the hive. Once exposed to sunlight adult beetles become active and show up out of the bee cluster on top bars and combs.
- Adult SHB will move quickly away from light, so it is important to make a quick visual scan as soon as the colony is opened.
- Use a variety of SHB traps to monitor the beetle. The most common traps are corrugated cardboard or plastic inserts and traps containing oil.
- If a suspected beetle is found, please contact your provincial apiculturist to report and validate your finding.

Management Treatment Options:

- Keep strong queen-right colonies at all times.
- Avoid conditions that might lead to colony stress such as brood diseases, mite problems, wax moth activity, failing queens, excessive swarming and over-supering.
- Extract supers of honey quickly to reduce the damage that SHB adults and larvae will do to standing, unprotected honey.

- Reduce the relative humidity to 50% or less in honey houses and hot rooms to inhibit SHB eggs from hatching.
- Remove honey, comb and cappings from around the honey house to minimize attractants for SHB.
- Use in-hive trapping devices to trap beetles. The traps vary in efficacy but provide some control of SHB adults.
- Consider using hygienic queens. Hygienic bees can remove beetle eggs and young larvae.

Chemical Treatment Options:

- Use GuardStar™ for ground treatment around colonies to kill pupating beetles
- Use Checkmite+™ under pieces of cardboard in colonies to kill adult beetles.

Name:

Tracheal Mite

Description:
The honey bee tracheal mite (HBTM) is a parasite that lives within the tracheae of adult honey bees. It feeds on the hemolymph (blood) of the bees and can cause severe losses in temperate climates.

Type:

Adult

Symptoms:

HBTM, *Acarpis woodi*, does not create specific visible symptoms in infested adult honey bees.

- Crawling and dead bees in front of hive.
- Poor spring colony population build-up.
- Colony may die in spring with very small cluster and large honey stores.

Monitoring Information:

The trachea must be examined under a microscope to diagnose HBTM. To determine tracheal mite infestation, collect 100 older bees from honey combs or the inner cover and place in 70% alcohol or winter windshield washing fluid. The bee sample can then be dissected and examined under a microscope, or sent to a bee diagnostic laboratory to determine the level of infestation.

Threshold**:** If the level of infestation is 10% or more in spring or fall, colonies should be treated.

Management Treatment Options:

- Use queens from genetically resistant stock.
- In temperate climate wrap bee colonies during winter.

Chemical Treatment Options:

- Formic acid. Follow label instructions closely.

Name:

Varroa Mite

Description:

The varroa mite is a serious honey bee parasite. The adult female mite has eight legs, is reddish-brown in color, flattened, oval and measures about 1 to 1.5 mm across. The damage caused to honey bees is severe including transmission of bee viruses that lead to colony kill. Detection and treatment are imperative to maintaining healthy bee colonies.

Type:

Adult

Symptoms:

The varroa mite, *Varroa destructor*, can be seen on adult bees, combs, in worker and drone brood and in hive debris.

At higher levels of infestation, indicators include:

- Crawling bees with deformed wings abandoning the hive.
- Parasitic mite syndrome (for details go to Parasitic Mite Syndrome).
- Mites on faces of combs, in drone brood and on bees.
- Colony often dies suddenly in late summer or fall.

Monitoring Information:

Monitor for mites in the early spring and late summer using one of the following methods:

- Mite Shake: Collect 300 adult bees from brood combs. Place bees in 70 % alcohol or winter windshield washing fluid. Shake vigorously to detach mites from bees. Use the Alberta *Varroa* shaker or a strainer to separate mites from bees. Count mites.
- Sticky boards: Place a sticky board 40 x 30cm (16 x 12in) under a screened bottom board. Leave for 72 hours. Remove and count fallen dead mites. Divide by three to get the number of mites dropped per day.

Thresholds based on monitoring method:

- Mite shake: Treat immediately if more than 7 mites/300 bees (approximately 3%).
- Sticky boards: Treat immediately if 10 or more dropped adult mites per day.

- Honey bees going into winter must have less than 1% mite infestation using the mite shake or fewer than 4 dropped mites/day on sticky boards before wintering.

Management Treatment Options:

- Use queens from stock that is mite tolerant. For example, use stock with hygienic traits.
- Remove capped drone brood.
- Interrupt bee brood cycle, for example by building nucleus colonies.

Chemical Treatment Options:

- Apivar™ is effective against *Varroa* mites. For best results, use one strip for every 5 frames or less of bees, in each brood chamber. Strips must be in contact with bees in the brood nest at all times. Remove strips after the 42 -56 day treatment period. For more instructions, please check the label.
- Apistan® was an effective miticide against *Varroa*. For best results, use one strip for every 5 frames or less of bees, in each brood chamber. Remove strips after the 42 day treatment period. For more instructions, please check the label.

Note: *Varroa* mites became resistant to Apistan® but a resistance reversal process is currently occurring. Therefore, you must test for Apistan® resistance to determine expected efficacy before using it.

- Checkmite+™: *Varroa* mites developed resistance to Checkmite+™ and so far there are no signs of resistance reversal to Checkmite+™.
- Formic acid in various formulations. Formic acid is effective against *Varroa* mites as well as Apistan® and Checkmite+™ resistant strains of *Varroa* mites. There are several registered formulations of formic acid. Check each product's label for detailed applications and conditions.
- Oxalic acid (Dripping Method): Apply 5 ml of 3.5% oxalic acid in sugar syrup on each bee space between frames occupied with bees. A maximum dose of 50 ml per colony can be applied. For more information, please check labels.
- Oxalic acid (Sublimation Method): sublimate 2 grams of oxalic dehydrate per colony. Follow instructions for application, method and safety according to the label. For more information, please check labels.
- Thymovar: For detailed application information, check the label.

Name:

Viruses

Description:

Honey bee adults are subject to infection by many viruses including: Deformed Wing Virus (DWV), chronic bee paralysis virus, acute bee paralysis virus, Kashmir bee virus and Israeli acute paralysis virus. Many of these viruses are associated with Varroa mites. The most commonly known virus is DWV; which can cause bee deformity. It can be transmitted and activated in developing and adult bees by Varroa mites.

Type:

Adult

Symptoms:

- Adult bees infected with DWV are small with shriveled and deformed wings. These bees are seen on combs and walking out of hive entrances unable to fly.
- Colonies infected with viruses may be slow to build up.
- Adult bees infected with various paralysis viruses show the following:
    - Lethargic, shaking, crawling, hairless and shiny bees.
    - Bees may be unable to fly.
    - Disjointed wings.

Monitoring Information:

- Monitoring, detection, and quantification of viruses can be done by taking adult bee samples and sending them to labs for molecular assays.

Management Treatment Options:

- Control *Varroa* mites.
- Use queens from stock genetically tolerant to *Varroa* mites.
- Keep strong, healthy bee colonies.

Chemical Treatment Options:

- No registered chemical treatments are available

Name:

Wasps and Hornets

Description:

The yellow jacket wasps and the bald-faced hornet can be nuisances to small honey bee colonies. Yellow jackets can be quite aggressive and invade colonies for honey and unguarded larvae. Hornets tend to be less aggressive and not so much of a problem.

The Asian predatory hornet is the most aggressive to honey bees and it also feeds on other insects. There is a concern it might expand to Canada so it is important for beekeepers to report any suspected finding of large hornet nests to the provincial apiculturist.

Type:

Hive

Symptoms:

- Many species of wasps and hornets are known to attack bee colonies such as: yellow jacket wasps (*Vespula* spp, *Dolichovespula arenaria),* baldfaced hornets (*Dolichovespula maculata),* and the Asian predatory hornet (*Vespa velutina* - Not currently found in Canada).
- Wasps and hornets can be found entering or inside bee colonies.
- Wasp nest can be found near the apiary.

Monitoring Information:

- Check apiaries for hornets, wasps and their nests.

Management Treatment Options:

- Maintain strong, healthy bee colonies.
- Have an experienced pest controller destroy any wasp or hornet nests found near the apiary.
- Place entrance reducers on small or weak colonies.
- Report any finding of suspected Asian hornets to the provincial apiculturist.

Chemical Treatment Options:

- Bait traps using sugar based attractants are <u>not recommended</u> due to attracting other beneficial insects and bees.
- There are no chemical control methods.

Name:

Wax Moth

Description:

There are two species of wax moth in Canada: the greater wax moth and the lesser wax moth. The greater wax moth, is usually responsible for the most damage. Wax moths can cause serious damage to bee boxes and combs. Wax moth larvae are elongated, cream-coloured to slightly golden grubs. They have three pairs of legs near the anterior and four pairs of less-developed prolegs toward the posterior. The wax moth larvae lack dorsal spines. They may be mistaken for small hive beetle larvae.

Type:

Hive

Symptoms:

Two species of wax moth are found in Canada: the greater wax moth (*Galleria mellonella)* and the lesser wax moth (*Achroia grisella*).

- Silk webbing on comb and cocoons stuck to frames.
- Boat-shaped gouges in wooden supers and frames.
- Moths, pupae and larvae in and around boxes, combs, bottom boards and lids.

Monitoring Information:

- Wax moths tend to be more of a problem in stored bee equipment than in live bee colonies. They have a preference for dark, older brood combs.
- Check combs for tunnels of silk and cocoons stuck to frames and boxes.
- Check weak, stressed colonies for wax moth infestation.
- A treatment threshold has not been established for the wax moth.

Management Treatment Options:

- Keep strong colonies to prevent wax moth damage in hives.
- Store dark brood comb separately from light brood and honey combs. This will lessen potential damage to light brood and honey combs.
- Place all comb on hives every year to allow bees to clean up any early infestations.
- Store equipment in sub-zero temperatures for at least a few hours to kill all life stages of the wax moth.
- Avoid conditions that might lead to colony stress such as brood diseases, mite problems and failing queens.

Chemical Treatment Options:
There is no chemical control agent registered for use against the wax moth in Canada.

HopGuard Registration application information

Rational to Support High Priority of HopGuard for Control of Varroa Mites (*Varroa destructor*) to Beekeeping and Minor Crops Pollination Industries

1. **Submission Contact Info.**

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2. Nature and Scope of the Problem

2.1 . Introduction

Recently, the honey bee (*Apis mellifera*) health in Canada has been challenged. Beekeepers have been reporting higher than normal winter losses of bee colonies. These reported winterkills are more than double the normal long term loss average of 15%. One of the main causes of colony losses in Canada is inadequate treatmentof Varroa mite (*Varroa destructor*) due to the spread of treatment-resistant varroa mites. This problem is compounded by limited options for managing those resistant mites. The threat that varroa mites pose to the beekeeping industry cannot be underestimated. These parasitic mites and resistant strains have been linked to high colony mortalities during winter (Scott-Dupree 1996, Currie et al., 2010; Guzman et al., 2010; Nasr, et. al., 2017). Moreover, the presence of vectored viruses in honey bee colonies most likely correlated with reported high colony mortality and the presence of the Colony Collapse Disorder in the USA (Cox-Foster *et al*., 2007; Highfield, et. al. 2009; de Miranda, et.al. 2012). For these reasons, varroa mite is regarded as the most economically important pest to honey bees around the world.

In recent years there is a wide spread treatment resistance to the registered synthetic acaricides, Apistan® (fluvalinate) and CheckMite^{+}™ (coumaphos). Registered alternative treatments such as formic acid, oxalic acid and thymovar have a variable range of efficacy. The efficacy of these alternatives is always dependent on colony size, presence of brood, and ambient weather conditions. Over all, these products do not give reliable control of mites. Consequently, Apivar® is the only option that Canadian beekeepers have to effectively control varroa populations. Apivar® is known to be not affected by certain weather conditions or bee colony status. It has been registered in Canada initially as emergency use and then full registration,

since 2008. Beekeepers have been using Apivar® as an effective control alternative to Apistan® and CheckMite+™.

The reliance on only one effective synthetic miticide (Apivar) and continuous use of the product since 2008 have raised serious concerns. If varroa mites develop resistance to Apivar®, The sustainability of the beekeeping industry and the ability to supply adequate numbers of healthy bees for crop pollination will be uncertain. The Canadian Honey Council, Canadian Provincial Apiculturists, Canada Standing Senate Committee on Agriculture and Forestry and Bee Health Roundtable members have identified that the need for effective options for varroa mite control is at the top of the priority list.

2.1. Scope of the Problem: Varroa mite and the development of treatment resistance

Varroa mites were first discovered in Canada in 1989. Because there were no registered controls for varroa mites, honey bee colonies with this mite in 1989 and 1990 were destroyed. In 1991, the emergency registration of the synthetic pyrethroid acaricide, Apistan® (fluvalinate) to control the varroa mite was granted. Apistan® was given full registration in 1993 (Clay 1996).

When varroa mites were first found in the United States, the slow industry response to the potential impact of the pest allowed it to spread before treatments like Apistan® became available (Wenner and Bushing 1996). It is believed that the enormous negative impact of varroa mite on honey bees was averted in Canada because of early registration and adoption of an effective mite control product like Apistan®.

Until early 2000, the varroa mite had been successfully controlled in Canada primrily using only Apistan®. To a lesser extent some beekeepers were using formic acid, but with mixed results. In 2001, a population

of varroa mites resistant to the active ingredient fluvalinate (Apistan®) was discovered in Canada. The development of strains of varroa mite resistant to fluvalinate had already been documented in a number of countries including the United States. Over the next four years, resistancetesting of varroa populations using the method described by Pettis, et. al. (1998), showed that fluvalinate-resistant varroa mites had become widespread across Canada.

In response to the impending threat that treatment-resistant varroa mites posed on the beekeeping industry in Canada, in 2002 PMRA granted an Emergency Use Registration (EUR) of CheckMite+™ Beehive Pest Control Strip (10% coumaphos) for the control of varroa mites in honey bee colonies in the affected provinces. Similar to the fluvalinate- resistant varroa mites, testing for coumaphos resistance showed that varroa mites had developed resistance to coumaphos as early as 2007 in some Canadian provinces.

In 2008, the occurrence of treatment-resistant varroa mites to both CheckMite+™ and Apistan® was considered widespread. In August 2008, at the request of Canadian Honey Council and provincial governments the PMRA approved the Emergency Registration of Apivar® for the control of varroa mite in honey bee colonies. In 2012 Apivar® was granted full registration for use in Canada.

Since 2008, Apivar has shown to have very good efficacy, often with control good enough that the colonies typically will need a single treatment. In some cases, alternative mite control options have been used as a supplementary treatment as needed. This practice is used to slow down the development of resistance as a part of resistance development management strategy.

The development of pest resistance to a control product is not a new phenomenon. Replacement of a single miticide (e.g. Apistan®) with another single miticide (e.g. CheckMite+™) has resulted in acceleration of resistance development. Once again the reliance on the use of a single miticide (e.g. Apivar®) for 8 or more consecutive years will eventually accelerate the rate of resistance development to this product.

Unfortunately, even in cases where producers were alternating between the limited number of control products (e.g. Apistan® or CheckMite+™) with formic acid and oxalic acid, once resistance was developed, the rotation did not provide enough time for "resistance- reversal" to occur. It appeared that rotation with treatments dependent on ambient temperature and bee colony status that yielded inconsistent efficacy rates might have also contributed to the spread of the resistant strains of mites. When treatment-resistant mites are not adequately controlled, the mites are allowed to reproduce and will disperse to neighboring colonies; Inadequate control of treatment resistant mites will promote the propagation of treatment resistant mites across the country. Consideration must be given to alternative miticides that have efficacy independent from ambient temperature.

3. A Potential Bio-pesticide for *Varroa destructor*: HopGuard2

3.1 . Hop- beta acid and HopGurad2 products

Hop-beta acids are naturally-occurring weak organic acids produced by hop plants (*Humulus lupulus* L.) (Jones et. al. 2003). They are readily available in extracts of the cones of hop plants. They are non-toxic to humans and they act as a bio-pesticide. They can effectively deter the feeding and egg-laying of plant-feeding mites (Jones et. al. 1996, 2003; DeGrandi-Hoffman et. al. 2012).

DeGrandi-Hoffman et. al. (2012) found that hop extracts can be used in bee colonies for the control of varroa mites. A delivery method was developed to apply the active ingredient. The end-use product is called HopGuard ® and consists of strips of cardboard impregnated with the formulated active ingredient(potassium salts of beta acids derived from hops). Treatment with HopGuard significantly increases varroa mite mortality (Rademacher and Harz, 2011; DeGrandi-Hoffman et. al. 2012; Probasco et. al., 2013; Vandervalk et. al., 2014; Nasr et. al. 2013; Nasr and Muirhead 2014). A revised formulation of HopGuard®, named HopGuard2 ®, was developed and tested. HopGuard2® provided mite control at the high end of the efficacy range based on Canadian studies (Nasr et. al. 2013). The reported efficacy ranged from 65% to 98.5%. Further studies conducted in Alberta showed that the efficacy of applying one and two applications of one strip of HopGuard2® for every 5 frames covered with bees in early summer was 34% and 79%, respectively. The HopGuard2 did not negatively impact the bees and brood production in treated colonies. Determination of beta acid residues in honey harvested from colonies that were treated with HopGuard2® strips, showed that negligible amounts were found in honey. Beta acids are natural acids found in many food products and they do not cause health concerns for humans at levels found in analyzed honey samples.

3.2 HopGuard2® as a potential Bio-pesticide fits Canadian needs

HopGuard2 ® has several properties that can fill the identified gap in varroa management practices. These properties are as follows:

a) Beta acids are the active ingredient in the HopGuard2®. They are non-toxic to humans. They are considered low risk-miticides (bio-pesticide) that can be safely used on crops.

b) HopGuard2® is a contact miticide that does not have cross resistance with other currently registered miticides for varroa control.

c) HopGuard2® is unlike currently registered organic acid (e.g. formic acid and oxalic acid) and essential oils (Thymovar) that require a certain range of ambient temperature and sometimes absence of brood to achieve high efficacy levels. The activity of HopGuard2® is independent of ambient temperatures, and is effective throughout the entire honey bee season. The activity of HopGuard2® does not require a specific window through the year for treatment or colony conditions such as the absence of brood.

d) HopeGuard2® treatment can be used at various times of the year (Probasco et. al., 2013; DeGrandi-Hoffman et. al., 2014) including during the honey flow. This is a major advantage of this product over other varroa mite treatments currently registered for use in Canada.

e) There are few pesticides that have low toxicity to honey bees. Treatment with HopGuard2® did not significantly increase adult honey bee mortality when the proper dose was applied in hives or in the laboratory (Rademacher and Harz, 2011; DeGrandi-Hoffman et. al., 2012; Nasr et. al., 2013; Probasco et. al., 2013).

f) The observed effects of HopGuard2® on colony population build up showed no significant difference between the untreated control colonies and the HopGuard- treated colonies (Probasco et. al., 2013).

g) Beta acid residues in honey extracted from colonies treated with HopGuard2® are insignificant.

h) HopGuard2® can be effectively used as an alternative Bio-miticide for the management of varroa mites resistant to treatment with Fluvalinate, Coumaphos.

i) HopGuard2® can be effectively used in an Integrated Pest Management (IPM) system to control varroa mite populations and manage treatment resistance by utilizing a combination of cultural, physical, and chemical management practices.

j) HopGuard® Has been a registered bio-pesticide for varroa control in bee colonies by the Environment Protection Agency (EPA) in the United States since 2015. Currently an amendment is submitted to replace HopGuard® with HopGuard 2 ® to modify the application method and improve the efficacy based on data from Canadian studies.

3. Economics of Not Adequately Controlling Varroa Mites

Canada has approximately 8,483 beekeepers and 672,094 honey bee colonies. The value of Canada's honey industry is estimated to be $176 million (Statistics Canada, 2013). The value of honey bees to Canada's agricultural economy, including pollination of crops, high value cash crops and minor crops, is estimated to be over $2 billion.

The economic losses associated with varroa mite are serious. In Canada, the long term average colony mortality is reported to be approximately 15% (Pernal 2007). The colony mortality in Canada ranged from 30%-35% from 2007- to 2010. The range of colony mortality decreased to 15-28% since 2011. The reduction in mortality is likely related to better control of varroa mites using Apivar. In 2011 and 2013 an extended period of cold in the spring and other reported exposure to pesticides resulted in higher than anticipated mortality.

Based on the above information, Canadian beekeepers lost 600,000 honey bee colonies between 2007 and 2009, which is equivalent to 100% of the colonies in Canada, mainly due to failure to effectively control varroa mites. As a result, beekeepers have incurred additional costs for purchasing or making up replacement colonies and loss of crop. A study conducted by Alberta Agriculture and Rural Development in 2007 revealed that the economic loss experienced by the Alberta beekeeping industry was estimated to be between $16.7 and $24.6 million as a result of increased colony losses from 15% to 30% (Chaudhary and Nasr, 2007). A recent study in Alberta also showed that the potential economic losses could be as high as $70 million/year due to high prices of bees, pollination services' fees and honey. In Canada, the estimated

economic impact of losing 30% of the honey bee colonies in one year was calculated to be valued at approximately $ 175 million per year.

Successive annual colony losses by Canadian beekeepers at levels exceeding long term averages are unsustainable. Development of apivar resistance in varroa mites will likely lead to high losses of bee colonies. This will decrease the number of healthy honey bee colonies available for honey production, and crop pollination This would be damaging to yields of certain horticultural crops that require pollination.

It is for those expected impacts when Apivar fails, we request from the Pest Management Centre- Minor Use Pesticide Program to consider HopGuard2® in the high priority list. Supporting HopGuard2® and expediting the registration as soon as all data is completed will enable beekeepers to have access to a new and effective bee protection tool. Thus the industry will become more sustainable, stay competitive, and be able to supply enough healthy bees for crop pollination.

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BetaTec® hop products

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July 18, 2017

Submission Coordination and Documentation Division
Pest Management Regulatory Agency
Health Canada
A.L. 6605E1
2720 Riverside Drive
Ottawa, ON K1A 0K9
Canada

Re: **Registration of HopGuard® II, a non-conventional pest control product – TGAI, MA and EP**

Dear Sir / Madam:

PMRA conducted a Pre-submission Consultation on HopGuard® on request from BetaTec Hop Products, Inc. in 2012 and provided BetaTec Hop Products, Inc. a PMRA Advice report dated May 15, 2012 (PMRA Report Number 2011-5047). This submission generally follows the guidance provided in this report.

This is the covering letter for the following three submissions:

1. Registration of Potassium Beta Resin (TGAI for HopGuard® II)
2. Registration of HopGuard® II Liquid (MA)
3. Registration of HopGuard® II (EP)

Together, this is the submission for the registration of TGAI, MA and EP for HopGuard® II as a high priority, non-conventional pest control product (per DIR2012-01) to control *Varroa* mites (*Varroa destructor*) in beekeeping and minor crops pollination. This submission comprises three packages:

1. e-Index Potassium Beta Resin (TGAI)
2. e-Index HopGuard® II Liquid (MA, manufacturing concentrate)
3. e-Index HopGuard® II (EP)

Following is a description / contents of each package:

Product names:	Potassium Beta Resin, HopGuard® II Liquid, HopGuard® II.
Relevant use-site category:	USC #8. Animals for Food Production, namely as treatment for honey bees in beehives, specifically as an acaricide for Varroa mites (*Varroa destructor*) in honey bees.
Related submissions:	e-Index Potassium Beta Resin (TGAI), e-Index HopGuard® II Liquid (MA), and e-Index HopGuard® II EP are being submitted as separate but related submissions
Active ingredient:	hop beta acids (present in technical hop extract as potassium salts, also internally referred to as Potassium Beta Resin)
Relevant history:	HopGuard® II TGAI (Potassium Beta Resin) was granted an unconditional Section 3 registration by U.S. EPA on 09/29/2015, EPA Registration Number 83623-3. HopGuard® Liquid (MA) was granted an unconditional Section 3 registration by U.S. EPA on 09/29/2015, EPA Registration Number 83623-1. HopGuard® II (EP) was granted an unconditional Section 3 registration by U.S.

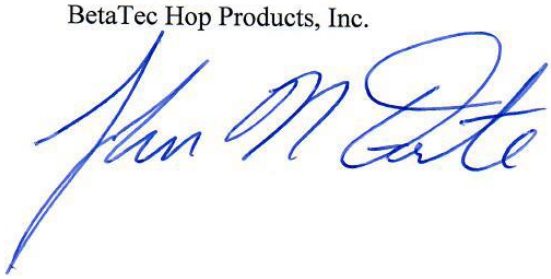
	EPA on 09/29/2015, EPA Registration Number 83623-2. Therefore, most of the data for this submission comes from the submission to EPA.
Letter of authorization:	Included in the e-Index. This letter designates David Hysert as our consultant and contact in Canada for this registration.
Letter of authorization:	Included in the e-Index. This letter authorizes PMRA to share data reviews with other countries.
Application forms (Form 6005):	Included in the e-Index.
Fee forms (Form 6011):	Included in the e-Index.
Fees:	The required fee has been provided (see explanation below).
SPSFs (Form 6003):	Three SPSFs (Statement of Product Specification Forms), one for EP, one for MA and one for TGAI, are included in the respective e-Indexes.
Draft label:	Included in the e-Index.
Index:	Included in the e-Index.
Scientific data:	Included in the e-Index. As stated above, most of the data for this submission comes from the submission to EPA.
Requests for waivers:	Included in the e-Index. Waivers from the submission to EPA are the primary waivers submitted here.
Overview document:	An overview spreadsheet format is included to facilitate navigating the submission.

This letter confirms that PMRA is authorized to speak to Dr. Tobias Laengle, Senior Biopesticides Coordinator, Pesticide Risk Reduction Program, Pest Management Centre, Agriculture and Agri-Food Canada on behalf of BetaTec Hop Products, Inc. regarding this submission.

Ms. Farmer, the Manager of the Pesticide Risk Reduction Program of AAFC-Pest Management Centre, has kindly provided the accompanying letter of support.

Please note that, as explained below, it is the position of BetaTec Hop Products, Inc. that HopGuard® II should be considered exempt from application fees as per the Pest Control Products Fees and Charges Regulations (dated April 1, 2017; SOR/2017-9) and the Fee Form (6011) was completed accordingly. The Pest Control Products Fees and Charges Regulations state that section 2 of the regulation (which covers fees) does not apply to " pest control product whose active ingredient is […] referred to in the Food and Drugs Act " as "[…] a flavouring preparation, natural extractive, oleoresin, seasoning or spice" (see section 1 (d) (ii) (D) of the regulations). The technical active ingredient of the HopGuard® II is a natural extractive of hops that falls into this category, and therefore the exemption should apply to Hop Guard® II.

Yours sincerely,
John Forte
Vice President
BetaTec Hop Products, Inc.



Beta Acid Resin

Safety Data Sheet

1. Identification of the Substance and of the Company	
1.1 Product Identifier:	**Beta Acid Resin**
1.2 Relevant Uses:	Food or feed additive
Supplier: Emergency Contact Details:	**Barth-Haas Group / John I. Haas, Inc.** Address: 5185 MacArthur Blvd., N. W., Suite 300, Washington D. C. 20016, USA.
2. Hazards Identification	
2.1 Classification:	Not classified (Regulation (EC) No. 1272/2008) Not classified
2.2 Label Elements	N/A (not classified)
2.3 Other Hazards:	Beta Acid Resin has not been fully tested. Ingestion of a large dose may cause irritation of mouth, throat and digestive tract. The product may
3. Components/Information on Ingredients	
Beta Acid Resin contains naturally occurring hop beta acids, uncharacterized soft resins and miscellaneous components extracted from hops (*Humulus lupulus*).	
4. First Aid Measures	
4.1 Description of First Aid Methods:	Inhalation: Not applicable Skin contact: Wash skin thoroughly with soap and water. Launder contaminated clothing before reuse. Eye contact: Flood the eye with plenty of water. If any symptoms
4.2 Most Important Symptoms and Effects	May cause irritation if in contact with eyes

4.3 Indication of Immediate Medical Attention	None known
5. Fire-Fighting Measures	
5.1 Extinguishing Media:	Carbon dioxide, dry powder and foam.
5.2 Special Hazards Arising from Substance:	None

5.3 Advice for	None
6. Accidental Release Measures	
6.1 Personal Protection:	Wear appropriate protective clothing – see Section 8.
6.2 Environmental	Do not discharge onto the ground or into watercourses.
6.3 Methods for	Scoop/shovel into disposal container. Disposein
7. Handling and Storage	
7.1 Precautions for Safe Handling:	Avoid excessive contact with product. Use appropriate protective clothing as indicated in Section 8. Wash hands after use
7.2 Conditions for Safe	Store at 15 – 25 °C (59 – 77 °F). Keep container closed. Store in original
7.3 Specific End Uses:	The substance is manufactured for use as a food or feed ingredient and for such uses is not subject to registration via REACH
8. Exposure Controls/Personal Protection	
8.1 Control Parameters:	Not applicable
8.2 Exposure Controls:	Engineering Controls: Not required. Eye/Face Protection: Safety eyewear should be worn if handling creates exposure potential. Hand Protection: PVC, rubber, latex or nitrile gloves.

9. Physical and Chemical Properties	
Appearance:	Solid Resin
Odour:	Slight hop aroma
Odour Threshold:	No data available
pH:	Not applicable – non-aqueous
Freezing Point:	No data available
Boiling Point:	No data available
Flash Point:	No data available
Evaporation Rate:	No data available
Flammability:	Non-flammable
Upper/Lower	N/A
Vapour Pressure:	No data available
Vapour Density:	No data available
Density:	No data available
Solubility in Water:	Insoluble
Partition Coefficient:	No data available
Auto-ignition	No data available

Decomposition	No data available
Viscosity at 20oC:	No data available
Explosive Properties:	None known
Oxidising Properties:	None known
10. Stability and Reactivity	
10.1 Reactivity:	No reactivity hazards known
10.2 Stability:	Stable under normal conditions
10.3 Possibility of Hazardous Reactions:	None known
10.4 Conditions to	Keep container closed when not in use
10.5 Incompatible Materials	None known
10.6 Hazardous Decomposition Products	None known
11. Toxicological Information	
11.1 Acute Toxicity:	No data available. Hops and hop extracts are generally recognized as safe (GRAS) for their intended use in accordance with US
11.2 Skin	No data available
11.3 Serious Eye	No data available
11.4 Respiratory or Skin	No data available
11.5 Germ Cell Mutagenicity:	No data available
11.6 Carcinogenicity:	No data available
11.7 Reproductive	No data available
11.8 STOT-Single	No data available
11.9 STOT-Repeated	No data available
11.10 Aspiration Hazard:	No data available
12. Ecological Information	
12.1 Toxicity:	No data available
12.2 Persistence and	No data available
12.3 Bio accumulative	No data available
12.4 Mobility in Soil:	Product will not readily dissolve in water.

12.5 Results of PBT and	No data available
12.6 Other Adverse	No data available
13. Disposal Considerations	
Product disposal:	Dispose of in accordance with all applicable local and
Container disposal:	Labels should not be removed from containers until they have been cleaned. Contaminated containers should not be treated as household waste. Containers should be cleaned using appropriate
14. Transport Information	
14.1 UN-Number:	Not hazardous for transport.
14.2 Shipping Name:	N/A
14.3 Transport Hazard	Not hazardous for transport.
14.4 Packing Group:	Not hazardous for transport.
14.5 Environmental	No data available.
14.6 Special	None known
15. Regulatory Information	
15.1 Safety, Health and Environmental Regulations:	No data available
15.2 Chemical Safety	No data available
16. Other Information	
The information in this safety data sheet is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. The information in this document is based on our present knowledge and should be used only as a supplement to information already in your possession concerning this product. It does not represent any guarantee of the properties of the	

HopGuard® Liquid

A mite control product made from a carbon dioxide extract of hops

This label is for the Manufacturing Concentrate (MA), HopGuard® Liquid

Contains the Technical Grade of Active Ingredient, Potassium Salts of Hop Beta Acids

FOR MANUFACTURING, FORMULATING OR REPACKAGING

GUARANTEE: Potassium Salts of Hop Beta Acids ……16.0 %

(measured as hop beta acids)

CAUTION - : May cause eye irritation. On contact flush eye with plenty of water; get medical attention. For further information, refer to Material Safety Data Sheet for this product.

PREVENT ACCESS BY UNAUTHORIZED PERSONNEL

READ THE LABEL BEFORE USING

REGISTRATION NO.: XYZ PEST CONTROL PRODUCTS ACT

NET CONTENTS – XY kg

LOT - Y00000

Manufacturing date: Month, DD, YYYY / Best if used by: Month, DD, YYYY

Store at 15 – 25 °C (59 – 77 °F)

Manufactured for BetaTec by John I. Haas, Inc., Yakima, Washington, **USA**

BetaTec Hop Products, 5185 MacArthur Blvd, NW. Suite 300, Washington DC, 20016

Tel : (202) 777-4800, Fax : (202) 777-4895

NOTICE TO USER: This pest control product is to be used only in accordance with the directions on the label. It is an offence under the Pest Control Products Act to use this product in a way that is inconsistent with the directions on the label. The user assumes the risk to persons or property that arises from any use of this product.

FIRST AID

IF IN EYES: Hold eyelids open and flush with a steady, gentle stream of water for 15- 20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control centre or doctor for advice.

IF ON SKIN OR CLOTHING: Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control centre or doctor for further treatment advice.

IF SWALLOWED: Call a poison control centre or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by poison control centre or doctor. Do not give anything by mouth to an unconscious person.

Treat symptomatically. Note to Physician: "Probable mucosal damage may contraindicate the use of gastric lavage".

PERSONAL PROTECTION EQUIPMENT (PPE): Applicators and other handlers must wear appropriate protective eyewear, such as face shield or goggles long sleeved shirt and long pants, waterproof gloves and shoes plus socks.

USER SAFETY RECOMMENDATIONS:

Users must:

- Wear chemical resistant gloves (e.g. nitrile) when handling the strips.

- Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.

- Remove clothing immediately if product soaks through. Then wash thoroughly and put on clean clothing.

- Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

DIRECTIONS FOR USE:

To be used only in the manufacture of a pesticide which is registered under the Pest Control Products Act.

DO NOT contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes.

STORAGE, DISPOSAL & SPILLS:

Because this is a Manufacturing Concentrate which is manufactured and used only in the USA, STORAGE, DISPOSAL & SPILLS are not of concern in Canada.

BetaTec® hop products

efficient by nature®

HOPGUARD® II

Étiquette d'un acaricide proposée pour le Canada

À utiliser dans les ruches pour lutter contre le varroa (*Varroa destructor*) présent sur les abeilles domestiques

MATIÈRES ACTIVES : EN POIDS

Sels de potassium d'acides bêta du houblon : …….. 16,0 %

(Mesurés sous la forme d'acides bêta du houblon)

AUTRES INGRÉDIENTS : .....84,0 %

TOTAL 100,0 %

DANGER

GARDER HORS DE LA PORTÉE DES ENFANTS

CONTENU NET : □ **12 languettes** HOPGUARD® II **par sachet**

□ **24 languettes** HOPGUARD® II **par sachet**

□ **48 languettes** HOPGUARD® II **par sachet**

□ **96 languettes** HOPGUARD® II **par sachet**

Chaque languette est pliée en deux et contient 4,0 grammes de sels de potassium d'acides bêta du houblon.

Produit fabriqué par :

Mann Lake Ltd

501 1st South

Hackensack, Minnesota, 56452, É.-U.

MISES EN GARDE

Risques pour les humains et les animaux domestiques

Danger : Corrosif. Cause des dommages irréversibles aux yeux. Éviter tout contact avec les yeux ou les vêtements. Porter (préciser la protection oculaire appropriée telle que des lunettes à coque, un écran facial ou des lunettes de sécurité). Se laver soigneusement les mains à l'eau et au savon après avoir manipuler le produit, avant de manger, de boire, de mâcher de la gomme, de fumer ou d'aller aux toilettes. Retirer et laver les vêtements contaminés avant de les réutiliser.

PREMIERS SOINS

EN CAS DE CONTACT AVEC LES YEUX : Maintenir les paupières écartées et rincer 15 à 20 minutes sous un filet d'eau continu. Le cas échéant, retirer les lentilles de contact après les 5 premières minutes et continuer de rincer. Appeler un centre antipoison ou consulter un médecin.

EN CAS DE CONTACT AVEC LA PEAU OU LES VÊTEMENTS : Retirer les vêtements contaminés. Rincer immédiatement la peau à grande eau pendant 15 à 20 minutes. Appeler un centre antipoison ou consulter un médecin pour obtenir des conseils sur le traitement.

EN CAS D'INGESTION : Appeler un centre antipoison ou consulter un médecin immédiatement pour obtenir des conseils sur le traitement. Faire boire lentement un verre d'eau à la victime si celle-ci est capable d'avaler. Ne pas faire vomir à moins d'avoir été avisé de procéder ainsi par le centre antipoison ou le médecin. Ne rien administrer par la bouche à une personne inconsciente.

Remarque à l'intention du médecin : « En raison des dommages probables aux muqueuses, le recours à un lavage gastrique pourrait être contre-indiqué. »

ÉQUIPEMENT DE PROTECTION INDIVIDUELLE (EPI) : Durant l'application ou toute autre manipulation, porter un équipement de protection oculaire tel qu'un écran facial ou des lunettes à coque, une blouse à manches longues et un pantalon long, des gants imperméables, des chaussures et des chaussettes.

RECOMMANDATIONS POUR LA SÉCURITÉ DE L'UTILISATEUR

Les utilisateurs doivent :

- porter des gants résistants aux produits chimiques (p. ex. en nitrile) pour manipuler les languettes;
- se laver les mains avant de manger, de boire, de mâcher de la gomme, de fumer ou d'aller à la toilette;
- enlever immédiatement tout vêtement imbibé de pesticide, puis se laver soigneusement et mettre des vêtements propres;

MODE D'EMPLOI

Traitement contre le varroa dans les colonies d'abeilles : Les languettes doivent être appliquées à raison d'une languette pour cinq cadres Langstroth normalisés couverts d'abeilles dans chaque chambre à couvain (par exemple, deux languettes pour dix cadres couverts d'abeilles dans chaque chambre à couvain).

N^{bre} de cadres couverts d'abeilles	≤ 5	6-10	11-15	≥ 16
N^{bre} de languettes HopGuard® II	1	2	3	4

Les languettes ne doivent être placées que dans les chambres à couvain (et non dans les hausses à miel). Les languettes doivent être dépliées et suspendues au-dessus d'une des chambres à couvain du centre, près du milieu du cadre, chaque moitié de la languette de part et d'autre du cadre. Si une deuxième languette est utilisée, la mettre dans la chambre à couvain, au centre de la grappe, en laissant au moins deux cadres de distance entre les languettes. Suspendre les languettes dans la chambre à couvain de manière à ce que les abeilles puissent se déplacer des deux côtés de la languette. Les languettes ne doivent être placées que dans les chambres à couvain (et non dans les hausses à miel). Ne pas poser les languettes sur le dessus des cadres. Les laisser 10 à 15 jours dans la colonie. Refaire, au besoin, jusqu'à deux applications lorsque la population d'abeilles s'accroît au printemps et qu'elle diminue à la fin de l'été et à l'automne.

Ne pas faire plus de quatre applications par année. Cette limite comprend toutes les applications faites à une colonie d'abeilles (généralement au printemps, à l'été et à l'automne). L'application des languettes devrait dépendre de la quantité de varroas observée dans la colonie. Les utilisateurs ne doivent pas recueillir le miel ni la cire des chambres à couvain, mais uniquement des hausses à miel. Pour obtenir des résultats

optimaux, appliquer les languettes HopGuard® II lorsqu'il n'y a que peu ou pas de couvain dans la ruche. Porter des gants résistants aux produits chimiques (p. ex. en nitrile) pour manipuler les languettes. **NE PAS réutiliser les languettes.**

Avertissement

Ne pas verser le liquide excédentaire restant dans le sachet de HopGuard® II directement sur les abeilles, car cela pourrait causer des dommages excessifs aux abeilles, au couvain et aux reines.

LUTTE CONTRE LA RÉSISTANCE

Les populations de varroas peuvent devenir résistantes aux pesticides. L'acquisition de résistance dépend à la fois de la fréquence et du taux d'application ou de la dose appliquée. Après une application, les ravageurs les plus sensibles meurent et ceux qui le sont moins survivent, s'accouplent avec d'autres survivants et se reproduisent. La majorité des descendants qui s'ensuivent héritent de la résistance parentale. Les applications additionnelles continuent de ne tuer que les sujets sensibles restants. Le recours prolongé à une seule classe d'acaricides ayant le même mode d'action ou à un seul acaricide sélectionnera les sujets résistants, lesquels seront dominants dans les populations d'acariens des générations subséquentes. Pour prévenir l'acquisition de résistance et conserver l'utilité de chacun des pesticides, il est indispensable d'adopter une stratégie de lutte efficace contre la résistance. Le mode d'action des acides bêta du houblon n'a pas encore été défini; cependant, il peut entraîner la mort par pénétration du mince exosquelette du ravageur et causer l'asphyxie.

Pour retarder l'acquisition de résistance :

- Si possible, utiliser les acaricides en rotation, ce qui réduira la pression de sélection comparativement à l'utilisation répétée du même produit, de produits ayant le même mode d'action ou appartenant à la même classe chimique. Dans le cas d'applications multiples,

utiliser chaque fois un produit ayant un mode d'action différent avant de revenir à un produit utilisé précédemment.

- Utiliser les acaricides en se basant sur les principes de la lutte antiparasitaire intégrée (LAI). Cela comprend l'identification adéquate du ravageur, la surveillance pour la localité, le seuil d'intervention économique particulier et les seuils de dommages économiques, la tenue des dossiers et l'utilisation de toutes les pratiques de contrôle existantes (culturales, biologiques et chimiques).

- Maximiser l'efficacité en suivant strictement le mode d'emploi fourni sur l'étiquette, dont le dosage et le calendrier des applications.

- Exercer une surveillance continue des populations traitées pour vérifier si une résistance à l'acaricide est apparue et signaler toute résistance soupçonnée aux spécialistes locaux en vulgarisation.

- Communiquer avec votre spécialiste local en vulgarisation pour obtenir des recommandations additionnelles au sujet de la résistance aux pesticides et de la lutte contre cette résistance ou des recommandations sur la LAI pour votre région en particulier.

- Pour en savoir plus ou signaler une résistance soupçonnée, communiquer avec votre spécialiste local en vulgarisation.

- Retirer les languettes qui sont toujours dans la ruche après 15 jours.

RESTRICTIONS

- À utiliser uniquement dans la ruche.
- La quantité utilisée par application ne doit pas dépasser une languette par groupe de cinq cadres couverts d'abeilles.
- Enlever les languettes restantes dans les ruches après 15 jours.
- Ne pas utiliser HopGuard® II plus de quatre fois par année.
- Ne pas verser le liquide restant dans l'emballage sur les abeilles, car cela pourrait causer des dommages aux abeilles, au couvain et aux reines.

ÉLIMINATION ET DÉCONTAMINATION

Éliminer les emballages et les languettes utilisées conformément aux exigences provinciales. Pour tout renseignement concernant l'élimination des produits inutilisés ou superflus, s'adresser au fabricant ou à l'organisme de réglementation provincial compétent. Les languettes HopGuard® II inutilisées, le matériel d'emballage et le liquide restant dans l'emballage ne sont pas des déchets dangereux selon l'EPA; ils doivent par conséquent être éliminés comme des déchets solides non dangereux, conformément à la RCRA (c.-à-d. mis dans un sac à déchets et éliminés dans un site d'enfouissement).

ENTREPOSAGE ET DURÉE DE CONSERVATION

Les languettes inutilisées doivent être entreposées dans un contenant hermétique, au frais et à l'abri de la lumière. Lorsqu'il est entreposé comme il se doit et utilisé avant la date de péremption, ce produit ne se dégradera pas de manière importante.

AVIS À L'UTILISATEUR

Ce produit antiparasitaire doit être utilisé seulement selon le mode d'emploi fourni sur l'étiquette. Toute utilisation non conforme au mode d'emploi constitue une violation de la *Loi sur les produits antiparasitaires*. L'utilisateur assume les risques que l'emploi de ce produit comporte pour les personnes ou la propriété.

HopGuard® Liquid

A mite control product made from a carbon dioxide extract of hops

This label is for the Manufacturing Concentrate (MA), HopGuard® Liquid

Contains the Technical Grade of Active Ingredient, Potassium Salts of Hop Beta Acids

FOR MANUFACTURING, FORMULATING OR REPACKAGING

GUARANTEE: Potassium Salts of Hop Beta Acids ……16.0 %

(measured as hop beta acids)

CAUTION - : May cause eye irritation. On contact flush eye with plenty of water; get medical attention. For further information, refer to Material Safety Data Sheet for this product.

PREVENT ACCESS BY UNAUTHORIZED PERSONNEL

READ THE LABEL BEFORE USING

REGISTRATION NO.: XYZ PEST CONTROL PRODUCTS ACT

NET CONTENTS – XY kg

LOT - Y00000

Manufacturing date: Month, DD, YYYY / Best if used by: Month, DD, YYYY

Store at 15 – 25 °C (59 – 77 °F)

Manufactured for BetaTec by John I. Haas, Inc., Yakima, Washington, **USA**

BetaTec Hop Products, 5185 MacArthur Blvd, NW. Suite 300, Washington DC, 20016

Tel : (202) 777-4800, Fax : (202) 777-4895

NOTICE TO USER: This pest control product is to be used only in accordance with the directions on the label. It is an offence under the Pest Control Products Act to use this product in a way that is inconsistent with the directions on the label. The user assumes the risk to persons or property that arises from any use of this product.

FIRST AID

IF IN EYES: Hold eyelids open and flush with a steady, gentle stream of water for 15- 20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control centre or doctor for advice.

IF ON SKIN OR CLOTHING: Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control centre or doctor for further treatment advice.

IF SWALLOWED: Call a poison control centre or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by poison control centre or doctor. Do not give anything by mouth to an unconscious person.

Treat symptomatically. Note to Physician: "Probable mucosal damage may contraindicate the use of gastric lavage".

PERSONAL PROTECTION EQUIPMENT (PPE): Applicators and other handlers must wear appropriate protective eyewear, such as face shield or goggles long sleeved shirt and long pants, waterproof gloves and shoes plus socks.

USER SAFETY RECOMMENDATIONS:

Users must:

- Wear chemical resistant gloves (e.g. nitrile) when handling the strips.

- Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.

- Remove clothing immediately if product soaks through. Then wash thoroughly and put on clean clothing.

- Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

DIRECTIONS FOR USE:

To be used only in the manufacture of a pesticide which is registered under the Pest Control Products Act.

DO NOT contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes.

STORAGE, DISPOSAL & SPILLS:

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BetaTec® hop products

efficient by nature®

HopGuard® Liquid

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Contains the Technical Grade of Active Ingredient, Potassium Salts of Hop Beta Acids

FOR MANUFACTURING, FORMULATING OR REPACKAGING

GUARANTEE: Potassium Salts of Hop Beta Acids ……16.0 %

(measured as hop beta acids)

CAUTION - : May cause eye irritation. On contact flush eye with plenty of water; get medical attention. For further information, refer to Material Safety Data Sheet for this product.

PREVENT ACCESS BY UNAUTHORIZED PERSONNEL

READ THE LABEL BEFORE USING

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LOT - Y00000

Manufacturing date: Month, DD, YYYY / Best if used by: Month, DD, YYYY

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BetaTec® hop products

efficient by nature®

Rational to Support High Priority of HopGuard for Control of Varroa Mites (*Varroa destructor*) to Beekeeping and Minor Crops Pollination Industries

4. Submission Contact Info.

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Canadian Honey Council and Provincial Apiculturists

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Prince Albert, SK. S6V 6G1

5. Nature and Scope of the Problem

5.1 . Introduction

Recently, the honey bee (*Apis mellifera*) health in Canada has been challenged. Beekeepers have been reporting higher than normal winter losses of bee colonies. These reported winterkills are more than double the normal long term loss average of 15%. One of the main causes of colony losses in Canada is inadequate treatmentof Varroa mite (*Varroa destructor*) due to the spread of treatment-resistant varroa mites. This problem is compounded by limited options for managing those resistant mites. The threat that varroa mites pose to the beekeeping industry cannot be underestimated. These parasitic mites and resistant strains have been linked to high colony mortalities during winter (Scott-Dupree 1996, Currie et al., 2010; Guzman et al., 2010; Nasr, et. al., 2017). Moreover, the presence of vectored viruses in honey bee colonies most likely correlated with reported high colony mortality and the presence of the Colony Collapse Disorder in the USA (Cox-Foster *et al*., 2007; Highfield, et. al. 2009; de Miranda, et.al. 2012). For these reasons, varroa mite is regarded as the most economically important pest to honey bees around the world.

In recent years there is a wide spread treatment resistance to the registered synthetic acaricides, Apistan® (fluvalinate) and CheckMite^{+}™ (coumaphos). Registered alternative treatments such as formic acid, oxalic acid and thymovar have a variable range of efficacy. The efficacy of these alternatives is always dependent on colony size, presence of brood, and ambient weather conditions. Over all, these products do not give reliable control of mites. Consequently, Apivar® is the only option that Canadian beekeepers have to effectively control varroa populations. Apivar® is known to be not affected by certain weather conditions or bee colony status. It has been registered in Canada initially as emergency use and then full registration,

since 2008. Beekeepers have been using Apivar® as an effective control alternative to Apistan® and CheckMite+™.

The reliance on only one effective synthetic miticide (Apivar) and continuous use of the product since 2008 have raised serious concerns. If varroa mites develop resistance to Apivar®, The sustainability of the beekeeping industry and the ability to supply adequate numbers of healthy bees for crop pollination will be uncertain. The Canadian Honey Council, Canadian Provincial Apiculturists, Canada Standing Senate Committee on Agriculture and Forestry and Bee Health Roundtable members have identified that the need for effective options for varroa mite control is at the top of the priority list.

2.1. Scope of the Problem: Varroa mite and the development of treatment resistance

Varroa mites were first discovered in Canada in 1989. Because there were no registered controls for varroa mites, honey bee colonies with this mite in 1989 and 1990 were destroyed. In 1991, the emergency registration of the synthetic pyrethroid acaricide, Apistan® (fluvalinate) to control the varroa mite was granted. Apistan® was given full registration in 1993 (Clay 1996).

When varroa mites were first found in the United States, the slow industry response to the potential impact of the pest allowed it to spread before treatments like Apistan® became available (Wenner and Bushing 1996). It is believed that the enormous negative impact of varroa mite on honey bees was averted in Canada because of early registration and adoption of an effective mite control product like Apistan®.

Until early 2000, the varroa mite had been successfully controlled in Canada primrily using only Apistan®. To a lesser extent some beekeepers were using formic acid, but with mixed results. In 2001, a population of varroa mites resistant to the active ingredient fluvalinate (Apistan®) was discovered in Canada. The development of strains of varroa mite resistant to fluvalinate had already been documented in a number of countries including the United States. Over the next four years, resistancetesting of varroa populations using the method described by Pettis, et. al. (1998), showed that fluvalinate-resistant varroa mites had become widespread across Canada.

In response to the impending threat that treatment-resistant varroa mites posed on the beekeeping industry in Canada, in 2002 PMRA granted an Emergency Use Registration (EUR) of CheckMite+™ Beehive Pest Control Strip (10% coumaphos) for the control of varroa mites in honey bee colonies in the affected provinces. Similar to the fluvalinate- resistant varroa mites, testing for coumaphos resistance showed that varroa mites had developed resistance to coumaphos as early as 2007 in some Canadian provinces.

In 2008, the occurrence of treatment-resistant varroa mites to both CheckMite+™ and Apistan® was considered widespread. In August 2008, at the request of Canadian Honey Council and provincial governments the PMRA approved the Emergency Registration of Apivar® for the control of varroa mite in honey bee colonies. In 2012 Apivar® was granted full registration for use in Canada.

Since 2008, Apivar has shown to have very good efficacy, often with control good enough that the colonies typically will need a single treatment. In some cases, alternative mite control options have been used as a

supplementary treatment as needed. This practice is used to slow down the development of resistance as a part of resistance development management strategy.

The development of pest resistance to a control product is not a new phenomenon. Replacement of a single miticide (e.g. Apistan®) with another single miticide (e.g. CheckMite+™) has resulted in acceleration of resistance development. Once again the reliance on the use of a single miticide (e.g. Apivar®) for 8 or more consecutive years will eventually accelerate the rate of resistance development to this product.

Unfortunately, even in cases where producers were alternating between the limited number of control products (e.g. Apistan® or CheckMite+™) with formic acid and oxalic acid, once resistance was developed, the rotation did not provide enough time for "resistance- reversal" to occur. It appeared that rotation with treatments dependent on ambient temperature and bee colony status that yielded inconsistent efficacy rates might have also contributed to the spread of the resistant strains of mites. When treatment-resistant mites are not adequately controlled, the mites are allowed to reproduce and will disperse to neighboring colonies; Inadequate control of treatment resistant mites will promote the propagation of treatment resistant mites across the country. Consideration must be given to alternative miticides that have efficacy independent from ambient temperature.

6. A Potential Bio-pesticide for *Varroa destructor*: HopGuard2

6.1 . Hop- beta acid and HopGurad2 products

Hop-beta acids are naturally-occurring weak organic acids produced by hop plants (*Humulus lupulus* L.) (Jones et. al. 2003). They are readily available in extracts of the cones of hop plants. They are non-toxic to humans and they act as a bio-pesticide. They can effectively deter the feeding and egg-laying of plant-feeding mites (Jones et. al. 1996, 2003; DeGrandi-Hoffman et. al. 2012).

DeGrandi-Hoffman et. al. (2012) found that hop extracts can be used in bee colonies for the control of varroa mites. A delivery method was developed to apply the active ingredient. The end-use product is called HopGuard ® and consists of strips of cardboard impregnated with the formulated active ingredient (potassium salts of beta acids derived from hops). Treatment with HopGuard significantly increases varroa mite mortality (Rademacher and Harz, 2011; DeGrandi-Hoffman et. al. 2012; Probasco et. al., 2013; Vandervalk et. al., 2014; Nasr et. al. 2013; Nasr and Muirhead 2014). A revised formulation of HopGuard®, named HopGuard2 ®, was developed and tested. HopGuard2® provided mite control at the high end of the efficacy range based on Canadian studies (Nasr et. al. 2013). The reported efficacy ranged from 65% to 98.5%. Further studies conducted in Alberta showed that the efficacy of applying one and two applications of one strip of HopGuard2® for every 5 frames covered with bees in early summer was 34% and 79%, respectively. The HopGuard2 did not negatively impact the bees and brood production in treated colonies. Determination of beta acid residues in honey harvested from colonies that were treated with HopGuard2® strips, showed that negligible amounts were found in honey. Beta acids are natural acids found in many food products and they do not cause health concerns for humans at levels found in analyzed honey samples.

6.2 HopGuard2® as a potential Bio-pesticide fits Canadian needs

HopGuard2 ® has several properties that can fill the identified gap in varroa management practices. These properties are as follows:

k) Beta acids are the active ingredient in the HopGuard2®. They are non-toxic to humans. They are considered low risk-miticides (bio-pesticide) that can be safely used on crops.

l) HopGuard2® is a contact miticide that does not have cross resistance with other currently registered miticides for varroa control.

m) HopGuard2® is unlike currently registered organic acid (e.g. formic acid and oxalic acid) and essential oils (Thymovar) that require a certain range of ambient temperature and sometimes absence of brood to achieve high efficacy levels. The activity of HopGuard2® is independent of ambient temperatures, and is effective throughout the entire honey bee season. The activity of HopGuard2® does not require a specific window through the year for treatment or colony conditions such as the absence of brood.

n) HopeGuard2® treatment can be used at various times of the year (Probasco et. al., 2013; DeGrandi-Hoffman et. al., 2014) including during the honey flow. This is a major advantage of this product over other varroa mite treatments currently registered for use in Canada.

o) There are few pesticides that have low toxicity to honey bees. Treatment with HopGuard2® did not significantly increase adult honey bee mortality when the proper dose was applied in hives or in the laboratory (Rademacher and Harz, 2011; DeGrandi-Hoffman et. al., 2012; Nasr et. al., 2013; Probasco et. al., 2013).

p) The observed effects of HopGuard2® on colony population build up showed no significant difference between the untreated control colonies and the HopGuard- treated colonies (Probasco et. al., 2013).

q) Beta acid residues in honey extracted from colonies treated with HopGuard2® are insignificant.

r) HopGuard2® can be effectively used as an alternative Bio-miticide for the management of varroa mites resistant to treatment with Fluvalinate, Coumaphos.

s) HopGuard2® can be effectively used in an Integrated Pest Management (IPM) system to control varroa mite populations and manage treatment resistance by utilizing a combination of cultural, physical, and chemical management practices.

t) HopGuard® has been a registered bio-pesticide for varroa control in bee colonies by the Environment Protection Agency (EPA) in the United States since 2015. Currently an amendment is submitted to replace HopGuard® with HopGuard 2 ® to modify the application method and improve the efficacy based on data from Canadian studies.

3. **Economics of Not Adequately Controlling Varroa Mites**

Canada has approximately 8,483 beekeepers and 672,094 honey bee colonies. The value of Canada's honey industry is estimated to be $176 million (Statistics Canada, 2013). The value of honey bees to Canada's

agricultural economy, including pollination of crops, high value cash crops and minor crops, is estimated to be over $2 billion.

The economic losses associated with varroa mite are serious. In Canada, the long term average colony mortality is reported to be approximately 15% (Pernal 2007). The colony mortality in Canada ranged from 30%-35% from 2007- to 2010. The range of colony mortality decreased to 15-28% since 2011. The reduction in mortality is likely related to better control of varroa mites using Apivar. In 2011 and 2013 an extended period of cold in the spring and other reported exposure to pesticides resulted in higher than anticipated mortality.

Based on the above information, Canadian beekeepers lost 600,000 honey bee colonies between 2007 and 2009, which is equivalent to 100% of the colonies in Canada, mainly due to failure to effectively control varroa mites. As a result, beekeepers have incurred additional costs for purchasing or making up replacement colonies and loss of crop. A study conducted by Alberta Agriculture and Rural Development in 2007 revealed that the economic loss experienced by the Alberta beekeeping industry was estimated to be between $16.7 and $24.6 million as a result of increased colony losses from 15% to 30% (Chaudhary and Nasr, 2007). A recent study in Alberta also showed that the potential economic losses could be as high as $70 million/year due to high prices of bees, pollination services' fees and honey. In Canada, the estimated economic impact of losing 30% of the honey bee colonies in one year was calculated to be valued at approximately $ 175 million per year.

Successive annual colony losses by Canadian beekeepers at levels exceeding long term averages are unsustainable. Development of apivar resistance in varroa mites will likely lead to high losses of bee colonies. This will decrease the number of healthy honey bee colonies available for honey production, and crop pollination This would be damaging to yields of certain horticultural crops that require pollination.

It is for those expected impacts when Apivar fails, we request from the Pest Management Centre- Minor Use Pesticide Program to consider HopGuard2® in the high priority list. Supporting HopGuard2® and expediting the registration as soon as all data is completed will enable beekeepers to have access to a new and effective bee protection tool. Thus the industry will become more sustainable, stay competitive, and be able to supply enough healthy bees for crop pollination.

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DACO 5.2

Report Title:

Use Description and Exposure Scenarios (Handler and Post-Application) for HOPGUARD® II Acaricide on Honey Bee Hives

Product: HopGuard® II

Active Ingredient: ***potassium salts of hop beta acids***

Authors: Jim Chaput, OMAFRA; Paul Kozak, OMAFRA; Jason Sproule, NSDA; and Medhat Nasr, AAF.

Date: January, 2017

Sponsor:

Agriculture and Agri-Food Canada Pest Management Centre

TABLE OF CONTENTS

Use Description and Exposure Scenarios (Handler and Post-Application) for HopGuard® II on Honey bee hives

SUMMARY

The following clothing and personal protective equipment is required during application and handling of the product:

- chemical resistant gloves;
- protective eyewear such as: face shield or goggles;
- long-sleeved shirt and long pants;
- shoes and socks;

An apiary is the location where hives are kept. A typical honey bee operation could contain approximately 40 hives on 1 acre of land. Apiary size will however, vary region to region. There are numerous beekeepers who keep only a few hives and instances where over a thousand hives may temporarily congregate to prepare for trucking to new locations. Given that treatment occurs to a small area within hives, there is no contact with the outside environment. For this reason, apiary size, and land area should have little impact to occupational exposure.

Assuming that it takes approximately 1-2 minutes for an applicator (typically only 1 person is needed) to treat one hive, the pesticide applicator may spend approximately 1 hour to treat the average apiary in its entirety. If visiting multiple apiaries over a typical 9 hour workday applicator exposure may be between 2.5 to 5 hours depending on hive and apiary configuration. 8 hours is an absolute worst case exposure scenario for applicators. While four (4) applications per year is the maximum allowed by proposed labeling, it is likely that three (3) applications in a year is a realistic scenario. If additional treatments are required, a rotation with an alternative miticide is likely. Thus the total exposure duration for HopGuard® II applications, based on a 10-15 day treatment period, to an average apiary is estimated at approximately 40-60 days per year. The potential for volatilization of active ingredient potassium salts of hop beta acids from the product packaging is insignificant.

Re-entry into treated hives is necessary to scout for pests and diseases, application of treatments for other pests and disease issues, provide protein supplement and sugar feeds, monitor queen health and replace queens as needed, harvest honey, or manage hive strength through splitting or addition of supers or exchange of frames of bees, brood or stored honey and pollen. In many apiaries, workers may enter every day to harvest honey, or to perform other operations. These activities involve minimal contact with treated portions of the hive. Most workers will use gloves for these tasks. A re-entry interval (REI) of 0 hours is proposed for honey bee hives. Specific REIs do not currently exist for other labeled varroa mite control products such as fluvalinate, formic acid or amitraz and given the reduced risk nature of HopGuard® II it is proposed that labeling remain consistent between products.

Based on the above information, use of HopGuard® II on Canadian apiaries under the proposed label requirements and current regulatory guidelines does not pose undue risk to workers applying the product or reentering treated hives to perform necessary work tasks.

INTRODUCTION

The purpose of this report is to fully describe the parameters and conditions for the use of HopGuard® II acaricide on honey bee hives and the human activity and exposure patterns associated with its use.

HANDLER (MIXER/LOADER/APPLICATOR) ACTIVITIES

Use Site Category

Livestock for food (USC 8)

Site of Application

HopGuard® II is intended to be used inside honey bee hives

Production System

The Langstroth hive design, which is the most common amongst North American beekeepers, is comprised of one or more vertically stacked wooden (sometimes stryofoam) boxes containing 8-10 removeable frames. Other designs that orient colonies in a more horizontal space are increasingly popular among small operations but still represent a very small fraction of the industry. Bees draw wax comb, a pattern of hexagonal cells, to fill the wooden and/or plastic frames. Lower boxes, known as brood chambers, are reserved for rearing brood. A single queen bee lays eggs in cells of the wax brood comb, and worker bees tend to the developing larvae and store pollen and nectar in the surrounding cells. Extraneous nectar is stored and ripened to honey in frames of honey comb located in the

upper boxes (a.k.a supers) from which honey can be harvested for human consumption. Once honeycomb is full, the bees will cap the open cells with wax at which point honey frames and supers may be removed for harvest. Honey is extruded from frames by gravitational or centrifugal force and filtered before sealing in jars, pails or barrels. In addition to honey production beekeepers may also manage their colonies for the production of additional honey bee colonies, or queen rearing. This involves removing frames of honey bee brood and live honey bees from one or more colony and assembling these to make up new colonies. In addition, many beekeepers are increasingly managing colonies for pollination services, whereby colonies are temporarily moved to a location adjacent to a flowering crop. In some cases, honey production may be secondary to pollination and production of additional honey bee colonies.

Nature of the Effects on Harmful Organisms

The active ingredient, potassium salts of hop beta acids works on contact with varroa mites (*Varroa destructor)*. Honey bees disperse the active ingredient throughout the hive during the course of grooming and direct body-to-body contact. The specific toxicological mechanism(s) are not well understood but hop beta acids have been shown to repel feeding and oviposition as well as reduce survival of adult varroa mites in hives and other mite pest species such as the two-spotted spider mite (*Tetranychus urticae* Kock) on plants in crop systems.

Formulation

HopGuard® II is a corrugated cardboard, mylar-backed strip moistened with potassium salts of hop beta acids in propylene glycol solvent and an emulsifier (polysorbate).

Details of Intended Use, Rate of Use and Rate of Active Ingredient

Each HopGuard® II strip contains 4.0 grams of potassium salts of hop beta acids. For honey bee hives, the recommended rate is one strip per five standard Langstroth frames of comb covered with bees in each brood chamber. In a single brood chamber hive the maximum number of strips would be 2, and 4 strips for a double brood chamber hive.

Size of Producing Areas

The following table shows the total size of the honey bee hive production system on which HopGuard® II is proposed for registration in Canada, as well as average size for a typical apiary.

Table 1: Honey bee hives in Canada

	Total Number of Hives in	**Smallest Number of Hives per**	**Largest Number of Hives per Apiary(2,3)**	**Average Number of Hives per Apiary(4)**
Honey bee hives	725,000	1	1,000	40

1. CAPA National Survey Committee & Provincial Apiculturists 2016. Canadian Association of Professional Apiculturists

   Statement on Honey Bee Wintering Losses in Canada.

2. An apiary may be defined as a location where any number of hives are kept.

3. In preparation for transport large numbers of hives may be temporarily gathered in marshalling yards and mite treatments may be applied in these locations.

4. The number of hives per apiary is dependent on nearby floral resources, time of year, management goals of the beekeeper and may vary substantially between regions.

Area Treated Per Work Day

Bee hives are typically restricted to small, defined areas. Although bees typically forage beyond the land occupied by the apiary (up to a 2.5 km radius from their hives), most apiaries are small, accommodating all hives on less than 1 acre of land. Beekeepers may operate any number of apiaries. Treatments for varroa are applied within the colony and there is no contact with the surrounding environment.

Treatment is recommended when mites exceed a treatment threshold which is based on mite population growth models and economic injury levels. Scouting may be based on a sample of hives within each apiary but treatment to all hives within the apiary operation will typically occur once the treatment threshold is exceeded. Such use practices are in accordance with Integrated Pest Management programs, as is the use of selective alternative miticides such as: Apivar®, Apistan®, Thymovar®, Oxalic acid and Formic acid.

Data concerning average numbers of hives per apiary in Canada is unavailable, and would likely be skewed by extremely small (one or two hive) operations, and may vary greatly by region. Instead, 40 hives could be considered a sustainable number of hives per apiary in most regions (Table 1). In observations of typical apiary operations application rates of 40 hives / hour are expected.

The typical workday for workers in a beekeeping operation is 8 to 9 hours. In a typical workday a worker may travel to multiple apiaries to treat hives as well as perform additional types of colony management. For medium to large size operations, applications may be the only activity performed on a given day and applicators work individually or in pairs. It is estimated that applicators may handle each strip for 20 seconds and each colony may receive between 1-4 strips. Given time spent travelling between apiaries and opening and closing of hives, the actual handling time of strips is likely limited from 2.5 to 5.0 hr per applicator per day. In small operations, beekeepers may perform various other

apiary tasks within the same day, further limiting time spent handling miticide strips. Hives are not usually re-opened for at least a few (3) days.

Assuming, as a worst case scenario, that one person treats 40 double brood chamber hives/hour, approximately 1440 strips per day totaling 8 hours of handling time.

Method of Application and Type of Equipment Used

HopGuard® II strips come ready to use. Application of strips requires cutting packaging and simple placement of strips directly into the hives. No mixing of the product with water or other solvents is necessary. No loading of the product into a sprayer or other mechanical applicator occurs.

Maximum Number of Applications and Their Timing

The HopGuard® II label will allow a maximum of 4 applications per season per colony. Strips remain in hives for 10-15 days. Hives are retreated as necessary, up to 2 times when bee population is increasing in spring and 2 times as populations are decreasing late summer and fall. This limit includes all applications to a bee colony (usually during spring, summer, and fall).

Individuals Involved in Mixing/Loading and Applying the Product

In small to mid-size apiary operations, the beekeeper is the individual applying the product. In the largest operations, a supervisor, foreman or worker might perform the application. Though loading of mechanical applicators is not required, strips from multiple packages may be pre-loaded into plastic totes at the start of the day.

Application Procedure

HopGuard® II will be packaged in laminated foil and film. Users must cut the package to remove the miticide strips. Folded strips must be opened and hung over one of the center brood frames near the middle of the comb with one-half of the strip on each side of the comb. If using a second strip, both strips are applied in the brood chamber in the center of the cluster, with a minimum distance of 2 frames between the strips. Strips are suspended in the brood chamber in a way that the bees can walk on both sides of the strips ensuring full contact of bees with the surface of the strip. Strips are to be placed only in the brood chamber (not in the honey super). Strips are not to be laid along the top of the brood frames or bottom board, or any other manner than prescribed above. Strips are left in the colony for 10-15 days.

Personal Protective Equipment and Clothing

The following clothing and personal protective equipment are required by product labeling for handling of the product including application and clean-up:

- chemical resistant gloves;
- protective eyewear such as: face shield or goggles;

- long-sleeved shirt and long pants;
- boots and socks;

These requirements are listed on the label under USER SAFETY RECOMMENDATIONS. In addition beekeepers typically wear full sleeve bee suits or jackets and veil which prevents touching their face.

POST-APPLICATION ACTIVITIES

Summary of Post Application Exposure

Re-entry into treated honey bee hives is necessary to perform the following types of activities:

- Removing HopGuard® II strips after 10-15 days
- Scouting for insects and diseases
- Employing other IPM strategies
- Harvesting honey
- Managing colony size
- Providing protein and sugar feed supplements

For honey bee hives, workers may enter every day to harvest the honey or perform routine management. At a minimum hives should be inspected every three weeks to scout for health issues and perform basic management activities. Meanwhile, most other activities need to be performed through most of the life of the hives. Often hive activities can be performed without direct contact of the surfaces to which HopGuard® was applied.

Most workers use gloves for re-entry activities; either chemical resistant gloves for phytosanitary reasons or leather bee gloves for protection from bee stings. Workers typically wear a long-sleeved shirt and long pants as standard clothing in the apiary. A re-entry interval (REI) of 0 hours is proposed for HopGuard® II use on honey bee hives.

Re-entry Activities

IPM Scouting

Most apiaries employ integrated pest management (IPM) programs. At least bi-weekly, scouts enter the hives to evaluate disease pressure and pest threshold levels. The scout may contact the wax comb in brood chambers and honey supers when handling the hives for closer inspection or for sampling, but contact is generally intermittent or incidental. In most cases it is possible to avoid direct contact with comb on which the HopGuard® II strips are placed. Secondary contact may occur from contact with bees that have contacted strips. Indirect contact from handling of combs which bees have distributed HopGuard® II over is likely.

Applying other pesticides

Application of other pest/disease control products after HopGuard® II application, occurs prophylactically or if disease or pest pressures meet a threshold level. However, applicators are expected to have very minimal contact with surfaces previously treated with HopGuard® II because (1) application optimally does not require or result in significant contact, and (2) applicators wear personal protective equipment which, at minimum, includes gloves, full length pants and sleeved shirt or jacket and veiled face protection.

Harvesting honey

Harvesting honey or placement of empty honey supers on hives typically does not require examination of brood chambers where HopGuard® II strips are located. Exposure is therefore

considered to be minimal as (1) contact with treated surfaces need not occur and (2) harvesters wear either personal protective equipment which, at minimum, includes gloves, full length pants and sleeved shirt or jacket and veiled face protection.

Splitting hives

Colonies in hives may be divided to manage the size of existing colonies or create new colonies/hives. Splitting requires removal and handling of brood combs including treated surface. If the split is made during the 10-15 day application period, handling of the HopGuard® II strip may occur. Workers are expected to contact the strips in a manner and time period similar to initial application. However, total exposure over the course of the workday is expected to be much less than initial application as the procedure of splitting hives will take substantially more time than re- handling of strips. Workers wear personal protective equipment which, at minimum, includes gloves, full length pants and sleeved shirt or jacket and veiled face protection.

Removal of HopGuard® II strips

Following the 10-15 day treatment period workers may remove miticide strips. Much of the active ingredient is expected to have been dissipated throughout the hive by this time. Furthermore bees may have significantly degraded miticide strips through chewing of the cardboard carrier material. Workers are expected to wear personal protective equipment which, at minimum, includes gloves, full length pants and sleeved shirt or jacket and veiled face protection.

Other activities

Other activities such as re-queening hives, removal of queen cells, managing colony growth, providing protein or sugar feed supplements are carried out as needed. These activities do require entering brood chambers and manipulation of brood frames. However, these activities are not anticipated to occur for a minimum of three days post application and contact is expected to be similar to IPM Scouting (see above).

Timing, Frequency and Duration of Re-entry Activities

A typical work day for beekeepers is 8 to 9 hours. Re-entry activities are constantly required in apiaries, thus workers often spend entire work days performing various re-entry activities. The following table is provided to represent what a maintenance program at typical apiaries might entail, in terms of frequency and duration of activities. It must be noted that because there is variability in apiary size within and between provinces, there is also variability in frequency and timing of the activities described.

Table 2: Frequency and Timing of Apiary Activities in Canada

System	Activity	Frequency	Timing*
Honey bee Hive	Scouting Honey harvest	Every 2 - 3 weeks Weekly	4-1 to 10-1 5-3 to 9-4 3-2 to 5-2
	Providing Feed	Every 1 – 2 weeks	and 8-2 to 10-2
	Requeening	Once / year	As needed; 4-1 to 8-3
	Supering	2 weeks	As needed; 5-1 to 9-3
	Splitting	1-2 times per year	5-1 to 7-2

* Indicates month and week as "[month number]-[week number]". E.g., 12-1 indicates December, week 1.

Workers Performing Re-entry Activities

Individuals conducting the re-entry activities listed above are males and females of age 18 and older. These people may be full-time or seasonal contract workers. Since apiary operations are typically active throughout the spring, summer and early fall, seasonal employees (6-8 months per year) are most common. 1-2 fulltime workers may be employed year-round. Family members are also often involved in operation of keeping bees.

Principal Sources of Exposure

In re-entry activities, exposure will be primarily from hand and forearm contact with treated combs in the brood chamber. Exposure is minimized by wearing of gloves and full sleeve clothing.

Personal Protective Equipment and Clothing

Most workers use leather bee gloves or chemical-resistant gloves for re-entry activities; scouts wear chemical-resistant gloves for phytosanitary reasons. Honey harvesters wear gloves for food safety reasons. All workers wear a long-sleeved shirt and long pants as standard clothing. In addition to this, workers may also wear a bee suit over the other items of clothing.

Re-entry Intervals

A re-entry interval of 0 hours, as is typical for in-hive miticide treatments, is proposed for honey bee hives.

BYSTANDER EXPOSURE

The levels of exposure of bystanders present outside the treatment area will be less than that experienced by the applicator or a worker, which has been deemed acceptable level. Bystanders rarely visit apiaries and would be strictly prohibited from entering areas where applications are being made, and would not have any extensive contact with the hives. Inhalation exposure is minimal due to the physical properties of the compound and the fact that applications are made to a very small area and placed within a contained space (inside of the honey bee colony) separated from the outside environment. Apiaries will typically be vacated once applications are completed. Due to the potential for honey bees to sting, bystanders are not common near apiaries and are not likely to approach the honey bee colonies.

Value Assessment Template

Note to Applicant: This is the value assessment template that outlines the information required to support an application to register a pesticide or to add a proposed use to a currently registered product. It is intended to assist applicants in the preparation of the Value Summary upon which the value assessment is based. This template should be used in conjunction with relevant guidance documents (Regulatory Directive 2013-03; Regulatory Proposal 2010-07; Memorandum on Tank Mixes, etc.), summary tables for efficacy or crop tolerance trials, and use history information templates, as appropriate. Applicants are strongly encouraged to request a pre-submission consultation with PMRA early in the process in order to obtain advice relevant to the content, organization, and quality of the information package.

The Value Summary document that results from completing this template should be loaded under DACO 10.1. A reference to the DACO number that contains information relevant for each section of the template is included (in italics). Please note that DACOs for microbial pesticides are preceded by the letter "M".

Value Summary.1

1. Introduction

1.1. Product Description ***(DACO 10.2.1; M1.1, M1.2, M1.3)***

Provide a description of the formulated product. Include information related to the following points:

- Product Name
  - **HopGuard® II**
- Registration number (if currently registered)
  - **Not currently registered**
- Active ingredient(s)
  - **Potassium salts of Hop Beta Acids**
- Guarantee: By Weight
  - Potassium Salts of Hop Beta Acids……..……..……16.0%
  - OTHER INGREDIENTS: ……..…..…..…… 84.0%

  TOTAL 100.0%
- Formulation type
  - **IF = Impregnated Fabric (from SPSF 6003). HopGuard® Liquid (MA) is impregnated on corrugated cardboard-Mylar strips.**

Provide a description of the active ingredient(s). Include information related to the following points:

- Active ingredient

- **Potassium salts of Hop Beta Acids**

- Chemical class
    - **Weak organic acids**
- Mode of action
    - **Currently unknown.**
- Site of action classification
    - **See mode of action section**
- Mechanism of action
    - **See mode of action section**
- Mechanism of selectivity
    - **See mode of action section**
- Absorption / translocation in plants
    - **Not applicable. See EPA environmental waivers.**
- Factors influencing availability, mobility, degradation and persistence in the environment.
    - **Not applicable. See EPA environmental waivers.**

1.2. Use Pattern *(DACO 10.2.1; M1.2)*

1.2.1. Registered Use Pattern

- Unregistered product. There is no label in Canada for HopGuard® II

1.2.2. Proposed Use Pattern / Amendments to Registered Use Pattern *(DACO 10.2.1; M1.2)*

- For Varroa mite control in bee colonies: Strips must be applied at a rate of one strip per five standard Langstroth frames covered with bees in each brood chamber (For example, two strips per ten frames covered with bees in each brood chamber).

No. frames covered with bees	≤ 5	6-10	11-15	≥ 16
No. of HopGuard® II strips	1	2	3	4

- Strips are to only be placed in the brood chamber (not in the honey super). Folded strips must be opened and hung over one of the center brood frames near the middle of the frame with one-half of the strip on each side of the frame. If using a second strip, the strips must be placed in the cluster with a minimum distance of 2 frames between the applied strips in a single brood box. If more than 2 strips are applied, the additional strips should be applied in the second brood box in the same manner. The strips should be suspended in the brood chamber in a way that the bees can walk on both sides of the strips to be exposed to the material.
- DO NOT lay the strips on top of the frames. DO NOT pour excess liquid onto frames. DO NOT re-use the strips
- Leave strips in the colony for a 10-15 day interval.

- Repeat as necessary, up to 2 applications when the bee population is increasing in the spring and when the population is decreasing in the late summer and fall.
- A maximum of four applications per year. This limit includes all applications to a bee colony (usually during spring, summer, and fall).
- Application of strips should be based on levels of Varroa mites observed in the colony and the number of frames covered with bees (cluster size).
- Users must not harvest honey and wax from the brood chambers, only from the honey supers.
- For optimal results, apply HopGuard® II when little to no brood is present in the hive.

- crop(s)/site(s):
    - Honey bee hives

- pest claims with an indication of level of control (e.g., control, suppression) for each pest:
    - Varroa mites, one application could provide an average efficacy of 45%, two consecutive applications applied 10-15 days apart could provide an efficacy of 80-95%

- product proposed for use (formulation and guarantee)
    - Impregnated Fabric (from SPSF 6003). HopGuard® Liquid (MA) is impregnated on corrugated cardboard-Mylar strips.
    - Guarantee By Weight

- Potassium Salts of Hop Beta Acids……16.0%
- OTHER INGREDIENTS: ….……...……..…….. 84.0%

TOTAL 100.0%

- application rate
  - Strips must be applied at the rate of one strip per five standard Langstroth frames covered with bees in each brood chamber (For example, two strips per ten frames covered with bees in each brood chamber).

No. frames covered with	≤ 5	6-10	11-15	≥ 16
No. of HopGuard® II strips	1	2	3	4

- adjuvant or any other spray solution additive (if any) and its rate
  - N/A

- application method (spray, granular, in-furrow, etc.)
  - Strips are only placed in the brood chamber (not in the honey super). Folded strips must be opened and hung over one of the center brood frames near the middle of the frames with one-half of the strip on each side of the frame. If using a second strip, apply the strips in the brood chamber in the center of the cluster with a minimum distance of 2 frames between the strips. If two strips are applied, the strips must be placed in the cluster with a minimum distance of 2 frames between the applied strips in a single brood box. If more than 2 strips are applied, the additional strips should be applied in the second brood box in the same manner. Suspend the strips in the brood chamber in a way that the bees can walk on both sides of the strips. Do not

lay the strips on top of the frames. Do not pour excess liquid onto the frames. Leave strips in the colony for a 10-15 day interval.

- number of applications per season or crop cycle, and interval between applications
  - Up to 2 applications when the bee population is increasing in the spring and when the population is decreasing in the late summer and fall. Leave strips in the colony for a 10-15 day interval.
  - A maximum of four applications per year. This limit includes all applications to a bee colony (usually during spring, summer, and fall).

- application timing relative to crop growth stage
  - When the bee population is increasing in the spring
  - When the population is decreasing in the late summer and fall.

- application timing relative to pest growth stage
  - When the Varroa mite population reaches the economic threshold of 1% or greater based on a washed sample of 300 bees
  - When there is a daily mite drop of 10 mites/day on a sticky board.

- pre-harvest interval

- N/A

- spray volume and concentration (as appropriate)

  - N/A

- nozzle type and spray quality if relevant to the proposed use (e.g., flat fan nozzle producing a medium spray)

  - N/A

- for rotational cropping claims, the appropriate interval after which a rotational crop can be planted

  - N/A

- General use directions, such as conditions that warrant the use of the higher or lower application rate, or shorter or longer application interval.

- Application of strips should be based on levels of Varroa mite observed in the colony. If Varroa mite levels are less than 1%, application should be stopped. If Varroa mite levels after the first application are above 1%, then a second application should be applied in the brood chamber, not in honey supers during the honey flow.

The proposed label text can be included in this section. Alternatively, proposed label text as it relates to specific claims can be presented in Sections 2.4 and 3.4 immediately preceding the information submitted to support the claims.

- Proposed label is presented in section 2.4 and 3.4

1.3. Description of the Pest Problem *(DACO 10.2.2; M10.4.2)*

Provide a description of the pest(s) proposed to be added to the label, including:

- common and Latin binomial name(s)
    - Common Name: Varroa mite
    - Latin binomial name*: Varroa destructor* (Truman and Anderson, 2000)

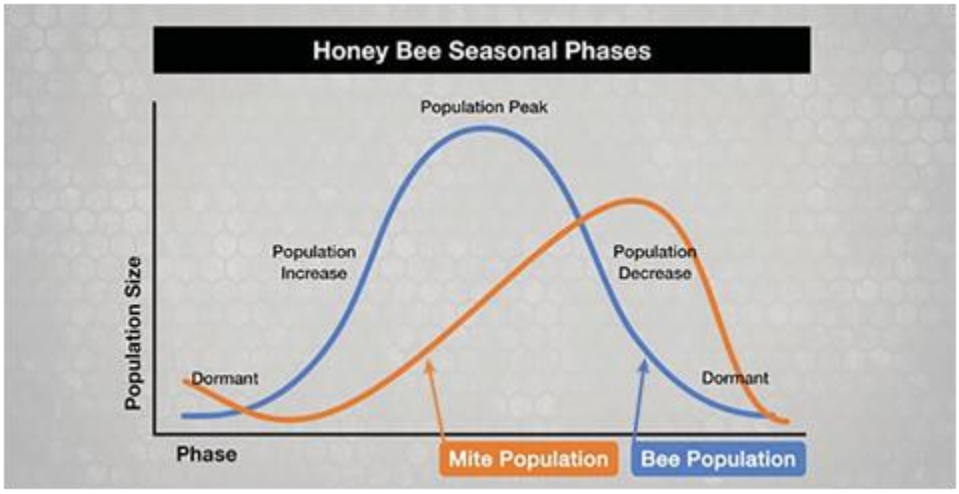
- the nature and severity of the damage to the crop(s) associated with the pest(s).
    - *Varroa destructor* is a natural parasite of the Asian honey bee, *Apis cerana*. These mites were accidently introduced into the Western honey bee, *Apis mellifera*. They were found in the USA

in the mid-1980's and in Canada in the early 1990's. They are external obligate parasites of worker and drone Western honey bees. They feed on the hemolymph of adult bees and developing pre-pupae and pupae. In this process, bee viruses such as the deformed wing virus (DWV), paralysis viruses and other viruses are transmitted to bees. Varroa also shortens the lifespan of honey bees and may even alter bee behaviour. A significant mite infestation will lead to the death of a honey bee colony within a year or two. Consequently, the *Varroa* mite is the parasite with the most pronounced economic impact on the beekeeping industry. It has also been considered a serious contributing factor to reported high colony mortality in Canada (Scott-Dupree 1996, Currie et al., 2010; Guzman et al., 2010, Hartman & Nasr, 2007, CAPA reports 2008-2016) and colony collapse disorder in the USA (Vanengelsdorp et. al. 2008).

- Relevant aspects of the pest(s) biology (e.g., life cycle) and its interaction with the crop should be included as appropriate. Complete descriptions of the pest species and their life cycles are not required.

    - Phoretic Varroa mites must enter bee brood cells before capping. In these cells, Varroa hides under the developing larvae that will become prepupae under the capping. The female mite that enters the cell is ready to reproduce. The female mite climbs onto the prepupae and begins feeding. Shortly thereafter, the mite lays its first egg on the surface of the cell wall. The egg is unfertilized and develops into a male mite. Subsequent fertilized eggs develop into female mites.

- During the Varroa feeding process on bee prepupae and pupae, mites transmit viruses. These viruses may cause damage to bees and one of the most telling signs of Varroa infestation is the occurrence of deformed wings caused by the deformed wing virus.
- Adult Varroa mites live up to 70 days. They reproduce on a 10-day cycle. Therefore, they have several reproduction cycles in their life. Overall, the Varroa population in a hive can multiply by about 12 times. High mite populations can cause crisis in the fall when summer bees and drones die, causing a quick crash and colony death in a short time (Fig. 1)



For details on Varroa and bee life cycle Check:

www.extension.org/pages/65450/varroa-mite-reproductive-biology

- If bee colonies are not treated in the spring, colony population build up will be compromised and honey yield will be reduced. Meanwhile, if bee colonies are not treated in the fall, winter bees will be impacted by Varroa infestation and high winter colony mortality is expected.
- During the active season, bees can drift to other adjacent colonies in an apiary, thereby, infecting the new colony. It is also known that bees from healthy colonies can rob honey from

failing colonies and bring Varroa to their own hives. These means of spreading Varroa are serious if mites are not controlled in infested bee colonies.

- Information on commercially acceptable levels of control, including economic thresholds, for each pest claim can be presented here.

  - The acceptable level of control is over 85%. It is advisable that a colony going to winter must have less than 1% Varroa mites using the wash method of 300 bees or less than 10 mites fallen on a sticky board in 24 hrs.

  - The economic threshold depends on the method of assessing Varroa mite infestation. The recommended methods for determining mite infestation include alcohol wash method and fallen mites on sticky boards.

  - The alcohol wash requires collecting 300 adult bees from bees on brood frames. The mites are separated from the bees using the Varroa mite shaker. If the number of collected mites is more than 7 mites/300 bees, then treatment is needed. This number is equivalent to a 3% infestation level as a correction factor of 1.9 mites/ sample added to the counted of fallen mites in the jar after shaking (Nasr et. al. 2017). For example if 7 mites fallen in the jar, total mites will be considered 9 mites. This will give 3% mite infestation in tested sample.

  - The sticky board method requires placing a sticky board in a modified bottom board to collect fallen mites. The installed sticky board is left for 3 days to collect fallen mites. All fallen mites are counted and the average fallen mites per day is calculated. The economic threshold for treatment using the sticky board is 10 mites/day.

  - Bee colonies must be sampled several times through the year to determine the infestation levels and detect mite population at critical times of the year in spring, late summer and fall.

Summary of Economic Thresholds.

Table 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 method) or natural *V. destructor* mortality per day.

Reference	Location, Time	Infestation (ether roll method)	Infestation (alcohol wash method)	Natural mortality (sticky board)
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%	
	Alberta, April		3%	>10 per day

2.	Nasr et al. 2008	Alberta, Fall	1%	10 per day

Product Efficacy ***(DACO 10.2, 10.2.3, 10.2.4; M10.1, M10.2.1, M10.2.2)***

This portion of the value summary presents all information related to efficacy. This may include experimental results from research trials, published scientific literature, scientific rationales, and use history information.

2.1. General Factors Affecting Product Efficacy

Describe any general factors that may influence product efficacy, such as:

- environmental and edaphic conditions
    - HopGuard® II is not affected by ambient temperature. It is unlike essential oils based products (i.e. Thymovar) and formic acid in that their efficacies depends on ambient temperature.

- application timing relative to crop or pest growth stage
    - HopGuard® II efficacy can be impacted by the presence of brood. If the product is applied in the spring and summer when too much brood is in the bee colonies, the efficacy decreases because mites confined in capped brood cells are not exposed to HopGuard® II.

- For high levels of mites and small population of bees: The activity of a small population of bees around the strips is very low. Therefore, the bees don't get enough material on their bodies to kill mites.
- During the honey flow when the bee population is too large (>50,000 bees/colony): The efficacy decreases due to low chances of bees contracting enough active ingredient from the HopGuard® II strips to kill mites on their bodies.

2.2. Supporting information from earlier formulations of the product or similar products

- HopGuard® was first developed as cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid. This formulation was registered in the USA.
- Research showed bees were able to chew the strips within a short period of time. Therefore, Impregnated Fabric (from SPSF 6003) was used for making new strips. HopGuard® Liquid (MA) is impregnated on these corrugated cardboard-Mylar strips.
- Guarantee: By Weight
    - Potassium Salts of Hop Beta Acids……..……16.0%
    - OTHER INGREDIENTS: ….……...…..……...…. 84.0%

    TOTAL 100.0%

- Data supporting efficacies and bridging to the newly developed HopGuard® II are presented in this section.

Summary Studies done during early stages USA Hop Beta Acid (HopGuard) on efficacy and bee toxicity

Study 1.

Reference: DeGrandi-Hoffman, et. al. 2012. The effects of beta acids from hop (*Humulus lupulus*) on mortality of *Varroa destructor* (Acari: Varroidae). Exp. Appl. Acarol. 58:407-421.

SITE	USDA-ARS Carl Hayden Bee Research Centre Apiary, Tucson, AZ
IN CHARGE OF EXPERIMENTS	Fabiana Ahumada-Segura
FORMULATIONS	Hop Beta Acid (HBA)
PRESENTATION	Cardboard strips (44.4 x 3.2 cm)
DOSAGE	2 strips per hive
Number of colonies	7 per treatment, 7 control
Number of Treatments	3
GROUPS	
• GROUP 1	Untreated control
• GROUP 2	2 strips for 21 days
• GROUP 3	2 strips replaced with 2 new strips after 14 days

Period of treatment	September 2010
Length of treatment	21 days
CONTROL TREATMENT	Apistan applied after 21 days

Results	Total mite drop per colony	Efficacy (%)
• GROUP 1 (control)	436	N/A
• GROUP 2	582	88.2
• GROUP 3	1,017	81.3

Damages or remarks	No damage was found in any colonies
Conclusions	Average mite drop after 2 days did not differ between groups 2 and 3 but was significantly higher than control.
Final comment	By day 21, significantly more mites were counted in control colonies compared with groups 2 and 3.

Study 2.

Rademacher: Unpublished data

SITE	Free University of Berlin, Germany
IN CHARGE OF EXPERIMENTS	Dr. Eva Rademacher
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips inserted vertically into the winter cluster
Number of colonies	22
Number of Treatments	3
GROUPS	
• GROUP 1	HopGuard strips on top of broodless frames, repeated after 6 weeks
• GROUP 2	HopGuard strips in winter cluster
• GROUP 3	Untreated controls
Period of treatment	October (Pre-field) - December 2010 (Main Group)
Length of treatment	December 6-23, 2010 (Main Group)

CONTROL TREATMENT	Oxuvar treatment
Results	Mite mortality %
• GROUP 1	82.6
• GROUP 2	91.3
• GROUP 3	6.8
Damages or remarks	Bee mortality was significantly higher than the control (7.16%). It is assumed that chemical drippings caused the mortality. During the October treatment (pre-field test), there was no bee mortality observed.
Conclusions	Late autumn treatments resulted in high mite kill with a mean efficacy of >90%.
Final comment	HopGuard could be a good product for Varroa control.

Study 3.

Reference: Rademacher and Harz. 2011. Application study: hop acids to control varroosis - colony trials. Assoc. of Inst. for Bee Res., Report of the 58th Seminar in Berlin, 29-31 March 2011. Apidologie 42: 788-789

SITE	Free University, Berlin, Germany
IN CHARGE OF EXPERIMENTS	Eva Rademacher
FORMULATIONS	HopGuard strip
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips hanging over the frames into the winter cluster
Number of colonies	10 treatment, 8 control; derived from nucei colonies
Number of Treatments	2
GROUPS	
• GROUP 1	Control
• GROUP 2	HopGuard
Period of treatment	Winter 2010-11
Length of treatment	17 days

CONTROL TREATMENT	Oxuvar

Results	Mite mortality (%)
• GROUP 1 (control)	7.8
• GROUP 2	93.5

Damages or remarks	Treatment was well tolerated.
Conclusions	Efficacy was significantly higher than the control. The highest mite mortality of HopGuard occurred within the first 24 h.
Final comment	This study supports the further investigation and approval of HopGuard as a miticide in Europe.

Study 4.

DeGrandi Hoffmann: Population growth of Varroa destructor (Acari: Varroidae) in commercial honey bee colonies treated with beta plant acids

Gloria DeGrandi-Hoffman • Fabiana Ahumada • Robert Curry • Gene Probasco • Lloyd Schantz

Received: 3 September 2013 / Accepted: 28 April 2014

SITE	Adee Honey Farms, Fresno, California, USA
IN CHARGE OF EXPERIMENTS	Gloria DeGrandi Hoffmann
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips
DOSAGE	2 strips per hive
Number of colonies	48 split hives
Number of Treatments	4
GROUPS	
• GROUP 1	1 application, inserted April
• GROUP 2	1 application in April; 3 applications in September
• GROUP 3	3 applications, inserted September

• GROUP 4	Untreated control
Period of treatment	April-September 2011
Length of treatment	48 hours
CONTROL TREATMENT	Normal mite drop was observed in the control group.

Results	Mites per 100 bees in September (Average)
• GROUP 1	1.25
• GROUP 2	1.3
• GROUP 3	2.5
• GROUP 4	4.0

Damages or remarks	No damage observed in the treated colonies.
Conclusions	Colonies with 3 treatments in September had significantly fewer mites per 100 bees than colonies with 1 application or the untreated controls
Final comment	No efficacy data were reported.

Study 5.

DeGrandi Hoffmann: Population growth of Varroa destructor (Acari: Varroidae) in commercial honey bee colonies treated with beta plant acids

Gloria DeGrandi-Hoffman • Fabiana Ahumada • Robert Curry • Gene Probasco • Lloyd Schantz

Received: 3 September 2013 / Accepted: 28 April 2014

SITE	Pendell Apiaries Stonyford, California, USA
IN CHARGE OF EXPERIMENTS	Gloria DeGrandi Hoffmann
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips
DOSAGE	1 strip per package; 2 strips per hive
Number of colonies	22 packages; developed in Langstroth hives
Number of Treatments	5
GROUPS	
• GROUP 1	1 application, inserted June 22
• GROUP 2	3 applications, inserted June 22, August 4, and October 11
• GROUP 3	2 applications, inserted August 4 and October 11

• GROUP 4	Package treated control
• GROUP 5	Untreated control
Period of treatment	June-October 2011
Length of treatment	48 hours
CONTROL TREATMENT	Normal mite drop was observed in the control group.

Results	Mites per 100 bees in October (Average)
• GROUP 1	14.8
• GROUP 2	3.0
• GROUP 3	5.0
• GROUP 4	16.0
• GROUP 5	10.7

Damages or remarks	No damage observed in the treated colonies.
Conclusions	Group 2 and 3 had significantly lower number of mites per 100 bees than the untreated controls.
Final comment	No efficacy data were reported.

Study 6.

Reference*:* Vandervalk, et. al. 2014. New Miticides for Integrated Pest Management of *Varroa destructor* (Acari: Varroidae) in Honey Bee Colonies on the Canadian Prairies**.** J. Econom. Ent. 107(6):2030-2036.

SITE	Crop Diversification Centre North, Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Lynae Vandervalk
FORMULATIONS	Hop Beta Acid (HBA)/HopGuard
PRESENTATION	Cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	- 1 HopGuard strip applied for every 4-5 frames of bees in each brood chamber. - 1 Thymovar Wafer per colony, applied on the top of the brood chamber - Two 40 ml 65% Formic acid in Dri-Loc pads applied weekly for 3 weeks - 1 Apivar strip for every 4-5 frames of bees
Number of colonies	8 per treatment, 8 control
Number of Treatments	5

GROUPS

- GROUP 1 Apivar
- GROUP 2 Formic Acid
- GROUP 3 HopGuard
- GROUP 4 Thymovar
- GROUP 5 Control (no treatment)

Period of treatment September 5-October 18, 2011

Length of treatment 42 days

CONTROL TREATMENT Oxalic acid sublimation (2 g/hive)

Results

Table. 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	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).
Damages or remarks	No damage was found in any colonies as a result of HopGuard treatment
Conclusions	HopGuard provided 42.96% efficacy against Varroa mites. The HopGuard had significantly lower efficacy in comparison to Apivar, Thymovar, and Formic acid.
Final comment	HopGuard single application did not provide an adequate control in fall.

Study 7.

Reference: Rademacher, et. al. 2015. The development of HopGuard as a winter treatment against *Varroa destructor* in colonies of Apis mellifera. Apidologie 46:748-759

SITE	Birkenwerder, Germany
IN CHARGE OF EXPERIMENTS	Eva Rademacher
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard (16% w/w beta acids)
DOSAGE	2 strips for small colonies; 4 strips for large colonies
Number of colonies	30
Number of Treatments	4
GROUPS	
• GROUP 1	2 strips in small colonies
• GROUP 2	Small control colonies
• GROUP 3	4 strips in large colonies
• GROUP 4	Large control colonies
Period of treatment	November 2011

Length of treatment	14 days
CONTROL TREATMENT	30 ml Oxuvar

Results	Mite mortality %
• GROUP 1	83.8
• GROUP 2	94.2
• GROUP 3	83.0
• GROUP 4	83.8

Damages or remarks	No damage was found in any colonies.
Conclusions	The mean efficacies did not exceed 90% and were not significantly different than the controls treated with oxalic acid.
Final comment	HopGuard has moderate efficacy against *V. destructor*.

Study 8.

Reference: Rademacher, et. al. 2015. The development of HopGuard as a winter treatment against *Varroa destructor* in colonies of Apis mellifera. Apidologie 46:748-759

SITE	Celle, Germany
IN CHARGE OF EXPERIMENTS	Eva Rademacher
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard (16% w/w beta acids)
DOSAGE	2 strips for small colonies; 4 strips for large colonies
Number of colonies	30
Number of Treatments	4
GROUPS	
• GROUP 1	2 strips in small colonies
• GROUP 2	Small control colonies
• GROUP 3	4 strips in large colonies
• GROUP 4	Large control colonies
Period of treatment	November 2011

Length of treatment	14 days
CONTROL TREATMENT	30 ml Oxuvar

Results	Mite mortality %
• GROUP 1	75.3
• GROUP 2	93.4
• GROUP 3	88.2
• GROUP 4	96.1

Damages or remarks	No damage was found in any colonies.
Conclusions	The mean efficacies did not exceed 90% and were not significantly different than the controls treated with oxalic acid. The highest mite mortality occurred within the first 72 hours.
Final comment	HopGuard has moderate efficacy against *V. destructor.*

Study 9.

Reference: Vandervalk, et. al. 2014. New Miticides for Integrated Pest Management of *Varroa destructor* (Acari: Varroidae) in Honey Bee Colonies on the Canadian Prairies. J. Econom. Ent. 107(6):2030-2036.

SITE	Crop Diversification Centre North, Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Lynae Vandervalk
FORMULATIONS	Hop Beta Acid (HBA)/HopGuard
PRESENTATION	Cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	- 1 HopGuard strip applied for every 4-5 frames of bees in each brood chamber. - 1 Thymovar Wafer per colony, applied on the top of the brood chamber - Two 40 ml 65% Formic acid in Dri-Loc pads applied weekly for 3 weeks - 1 Apivar strip for every 4-5 frames of bees
Number of colonies	8 per treatment, 8 control
Number of Treatments	5
GROUPS	

- GROUP 1 Apivar
- GROUP 2 Formic Acid
- GROUP 3 HopGuard
- GROUP 4 Thymovar
- GROUP 5 Control (no treatment)

Period of treatment May 7 - June 18, 2012: Finishing treatment removed July 31, 2012

Length of treatment 42 days

CONTROL TREATMENT Apivar strips (1 strip per 5 frames covered with bees)

Results

Table. 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®	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).

Damages or remarks	No damage was found in any colonies as a result of HopGuard treatment but damaged was found as a result of Thymovar treatment
Conclusions	HopGuard provided 43.56% efficacy against Varroa mites despite it was applied 3 times, 10 days apart. The HopGuard had significantly lower efficacy in comparison to Apivar, Thymovar, and Formic acid. Bees were able to chew the strips within 5 days of treatments
Final comment	HopGuard application did not provide an adequate control in spring

Study 10.

Reference: Nasr, et. al. 2013. An effective improved application method of HopGuard for Varroa Control in Canada. Not published.

SITE	Crop Diversification Centre North, Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Medhat Nasr
FORMULATIONS	Hop Beta Acid (HBA)/HopGuard strips
PRESENTATION	Cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	- 15 g of liquid HopGuard in a Petri dish replaced every 10 days, 2 applications total - 15g of liquid HopGuard and 2.5 g of thymol powder in a Petri dish replaced every 10 days, 2 applications total - 2.5 g of thymol powder in a Petri dish replaced every 10 days, 2 applications total - 4 HopGuards strips* in a screened frame replaced every 5 days, 4 applications total - 4 HopGuard strips* that were incubated for 3 days at 25°C replaced every 5 days, 4 applications total

- 4 HopGuard strips* that were incubated for 5 days at 25°C replaced every 5 days, 4 applications total
- 4 HopGuard strips* replaced every 5 days, 4 applications total
- Control (no treatment)

*2 strips per box

Number of colonies 5 per treatment, 5 control

Number of Treatments 8

GROUPS

- GROUP 1 15g liquid HopGuard
- GROUP 2 15g liquid HopGuard + 2.5 g thymol powder
- GROUP 3 2.5 g thymol powder
- GROUP 4 Screened HopGuard
- GROUP 5 3 day incubated at 25°C HopGuard
- GROUP 6 5 day incubated at 25°C HopGuard
- GROUP 7 HopGuard
- GROUP 8 Control (no treatment)

Period of treatment August 7-September 3, 2013

Length of treatment	20 days
CONTROL TREATMENT	1 Apivar strip per 5 frames of bees for 42 days

Results	Average ± STD Efficacy (%)
• GROUP 1	10.8 ± 3.7
• GROUP 2	9.4 ± 2.2
• GROUP 3	42.2 ± 14.6
• GROUP 4	9.9 ± 2.0
• GROUP 5	11.3 ± 5.1
• GROUP 6	20.5 ± 4.2
• GROUP 7	56.7 ± 16.9
• GROUP 8	10.7 ± 4.0

Damage or Remarks	No damage was found in any colonies as a result of HopGuard treatment
Conclusions	High mite mortality was seen 1-3 days after application. The low efficacy for screened strips confirms that HopGuard is a contact miticide. Strips dried quickly and were then chewed by the bees.
Final comment	HopGuard did not adequately control Varroa, possibly due to the strip design.

2.3. Requirement for Adjuvants

If an adjuvant is proposed to be used with the product, present information in this section to demonstrate why the adjuvant is required, or under what circumstances it is required. This information may include experimental results from research trials, published scientific literature, scientific rationales, and use history information.

If more than one adjuvant is proposed to be used with the product, present information in this section to demonstrate the similarity or interchangeability of the adjuvants, when used in combination with the product.

Experimental results from trials that evaluated the product both with, and without the inclusion of an adjuvant in the same trial should be presented in this section using a format similar to that presented below in Section 2.4 Support for Proposed Claims.

- N/A

2.4 Support for Proposed Claims *(DACO 10.2, 10.2.3., 10.2.3.3, 10.2.3.4, 10.2.4; M10.1, M10.2.1, M10.2.2)*

Each claim should be identified (i.e., each application timing, method of application, pest, tank mix, etc.), and the approach and information used to support each of these claims, with respect to efficacy, should be clearly indicated.

Claims may be organized in subsections by pest, application timing/method, etc., whichever is best suited to the specific claims being proposed.

Each subsection should present all the various types of information available (i.e., experimental results from research trials, published scientific literature, scientific rationales, and use history information) to support the claims within the subsection. It is important to note that this section of the value summary consists of a synopsis of all supporting efficacy information and not a compilation of the individual reports. Copies of individual reports should be submitted as a separate component of the value information package.

When research trials are presented, a summary of the experimental results as they relate to the proposed claims should be included. The applicability of factors such as application rate and timing, application method, number of applications, application interval, etc., to the proposed use pattern should be explained. Inconsistencies between the test parameters and the proposed use relating to product formulation, location of the trials, assessment parameters, expected results, or other factors should be justified. Individual trial reports should be included in the value package.

When published scientific literature is presented, an explanation of the relevance of the scientific paper/s to the proposed use should be provided. A comparison of the proposed use pattern and the use pattern employed in the studies should be included. All units should be expressed in metric to enable a direct comparison of the application rates. If the products tested in the study have equivalent products registered in Canada, they should be identified. Any unexpected results should be discussed. A copy of the published articles should be included in the value package.

When use history information is presented, a summary of the information provided in the templates should be presented. This should include (1) a brief explanation of the similarities of the proposed use pattern and the registered use pattern in the foreign jurisdiction, (2) information on the efficacy and crop tolerance profile of the proposed use, and (3) validation information describing the source of the information and the extent of their direct experience with the product.

When scientific rationales are presented, a clear explanation of the basis for support of the proposed use should be provided. It should contain the appropriate level of detail to enable a conclusion regarding the proposed claims to be made.

Regardless of the type of information submitted, the applicant should provide an explanation if the use pattern in the supporting information submitted differs from the proposed use pattern.

The subsection should finish with the presentation of an overall conclusion made on the basis of an integration of all the types of information presented to support the claim.

Study 1.

Reference: Nasr, et. al. 2013. An effective improved application method of HopGuard for Varroa Control in Canada. Not Published.

SITE	Crop Diversification Centre North, Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Medhat Nasr
FORMULATIONS	HopGuard and HopGuard II
PRESENTATION	- HopGuard: Cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid - HopGuard II: Corrugated cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	4 strips per hive for both HopGuard and HopGuard II
Number of colonies	6 per treatment, 6 control
Number of Treatment	5
GROUPS	
• GROUP 1	4 HopGuard strips replaced every 5 days, 3 times
• GROUP 2	4 HopGuard II strips applied once to colonies
• GROUP 3	4 HopGuard II strips applied twice replaced after 10 days

• GROUP 4	4 HopGuard II strips applied 3 times, replaced every 5 days
• GROUP 5	Control
Period of treatment	September 30 – October 21, 2013; Finishing treatment (Apivar) removed November 24, 2013
Length of treatment	21 days
CONTROL TREATMENT	Apivar Strips (1 strip per 5 frames of Bees)
Results	Alberta Efficacy %
• GROUP 1	90
• GROUP 2	73
• GROUP 3	96
• GROUP 4	98
• GROUP 5	18
Damages or remarks	No damage was found in any colonies
Conclusions	2 Applications of HopGuard II regardless of dose was very effective at controlling mites with an efficacy of 96% and 97% when applied twice and 98% when applied 3 times. There was no significant difference between 2 and 3 applications of HopGuard II and it efficacy as a miticide.
Final comment	2 applications of HopGuard II at the recommended dose of 1 strip per 5 frames of bees were effective at controlling mites in the fall.

Study 2.

Reference: Nasr, et. al. 2013. An effective improved application method of HopGuard for Varroa Control in Canada. Not Published.

SITE	Saskatchewan Ministry of Agriculture, Prince Albert, Saskatchewan, Canada
IN CHARGE OF EXPERIMENTS	Graham Parson and Geoff Wilson
FORMULATIONS	HopGuard I and HopGuard II
PRESENTATION	- HopGuard: Cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid - HopGuard II: Corrugated cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	4 strips per hive for both HopGuard and HopGuard II
Number of colonies	6 per treatment, 6 control
Number of Treatment	3
GROUPS	
• GROUP 1	4 HopGuard strips replaced every 5 days, 3 times
• GROUP 2	4 HopGuard II strips applied once to colonies
• GROUP 3	4 HopGuard II strips applied twice replaced after 10 days

Period of treatment	October 3-24, 2013
Length of treatment	21 days
CONTROL TREATMENT	Apivar Strips (1 strip per 5 frames of Bees)

Results	Saskatchewan Efficacy %
• GROUP 1	92
• GROUP 2	92
• GROUP 3	97

Damages or remarks	No damage was found in any colonies
Conclusions	2 Applications of HopGuard II regardless of dose was very effective at controlling mites with an efficacy of 96% and 97% when applied twice and 98% when applied 3 times. There was no significant difference between 2 and 3 applications of HopGuard II and it efficacy as a miticide.
Final comment	2 applications of HopGuard II at the recommended dose of 1 strip per 5 frames of bees were effective at controlling mites in the fall.

Study 3.

Ahumada: Unpublished data

SITE	USDA, Tucson Arizona, USA
IN CHARGE OF EXPERIMENTS	Fabiana Ahumada
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard liquid
DOSAGE	2 strips per brood chamber
Number of colonies	16
Number of Treatments	1
GROUPS	
• GROUP 1	1 application of HopGuard II
Period of treatment	Autumn 2013
Length of treatment	14 days
CONTROL TREATMENT	Sugar shake method
Results	Mite mortality %

- GROUP 1 86

Damages or remarks	No damage was found in any colonies
Conclusions	This was an exploratory study, but it showed a high efficacy for only a 14 day treatment. The honey samples did not contain detectable residues of hop beta acids.
Final comment	The results are encouraging.

Study 4.

Reference: Nasr et. al. HopGuard II Under-Over Dosage and Application Experiment, Summer 2014 – Not Published

SITE	Crop Diversification Centre North , Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Medhat Nasr
FORMULATIONS	Hop Beta Acid (HBA)- HopGuard II
PRESENTATION	Corrugated cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	- Half dose: ½ a strip of HopGuard II for every 5 frames of bees - Recommended dose: 1 strip of HopGuard II for every 5 frames of bees - One and one-half dose:1.5 strips of HopGuard II for every 5 frames of bees - Double dose: 2 strips of HopGuard II for every 5 frames of bees
Number of colonies	6 per treatment, 6 control
Number of Treatment	8
GROUPS	
• GROUP 1	Half dose applied once.

- GROUP 2 Half dose applied twice, 10 days apart
- GROUP 3 Recommended dose applied once
- GROUP 4 Recommended dose applied twice, 10 days apart
- GROUP 5 One and one-half dose applied once
- GROUP 6 One and one-half dose applied twice, 10 days apart
- GROUP 7 Double dose applied once
- GROUP 8 Control (no treatment)

Period of treatment
- GROUP 1,3,5,7 – June 16 to June 26, 2014, Finishing treatment (Apivar) removed August 7, 2014
- GROUP 2,4,6,8, - June 16 to July 6, 2014, Finishing treatment removed August 18, 2014

Length of treatment
- GROUP 1,3,5,7 - 10 days
- GROUP 2,4,6,8 - 21 days

CONTROL TREATMENT: Apivar Strips (1 strip per 5 frames of Bees)

Results: Efficacy %

- GROUP 1 33
- GROUP 2 72
- GROUP 3 34

- GROUP 4 79
- GROUP 5 36
- GROUP 6 65
- GROUP 7 46
- GROUP 8 22

Damages or remarks No damage was found in any colonies

Conclusions 2 applications of HopGuard II at any dose provided better mite control than a single application. There was no significant difference between the half dose and recommended dose when applied twice at 72% and 79% efficacy.

Final comment 2 applications as opposed to one of HopGuard II are significantly more effective at controlling Varroa mite populations. However, the 2 applications did not provide a high enough efficacy to be considered an effective miticide in the summer.

Study 5.

Theophilidis: Unpublished data

SITE	Aristotle University of Thessaloniki, Greece
IN CHARGE OF EXPERIMENTS	George Theophilidis
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips, hanging over frames 3/4 and 7/8, diagonally opposite each other
Number of colonies	8 per treatment, 4 control
Number of Treatments	4
GROUPS	
• GROUP 1	2 strips removed after 28 days
• GROUP 2	2 strips, replaced after 28 days, removed 28 days later
• GROUP 3	2 strips, replaced after 14 days, removed 14 days later
• GROUP 4	Untreated control: 2 blank strips removed 28 days
Period of treatment	Autumn 2014
Length of treatment	28 days

CONTROL TREATMENT	2 x 25 gram treatments of Apiguard with 1 week interval followed by 2 strips Apistan for 3 weeks and one application of oxalic acid when colonies became broodless
Results	Mite mortality %
• GROUP 1	78.84
• GROUP 2	91.95
• GROUP 3	87.12
• GROUP 4	10.14
Damages or remarks	No damage was found in any colonies
Conclusions	HopGuard II provided a significantly higher efficacy than the control. Two applications provided the highest efficacy.
Final comment	HopGuard II could be a good product for Varroa control.

Study 6.

Ahumada: Unpublished data

SITE	Texas and Dakota, USA
IN CHARGE OF EXPERIMENTS	Fabiana Ahumada
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard liquid
DOSAGE	2 strips per brood chamber
Number of colonies	180
Number of Treatments	3
GROUPS	
• GROUP 1	1 application, spring and autumn
• GROUP 2	2 applications at 14 day interval, spring and autumn
• GROUP 3	Control- alternative acaricide treatment
Period of treatment	Spring and autumn 2015
Length of treatment	28 days for both spring and autumn
CONTROL TREATMENT	Apivar (Amitraz) treatment

Results	Mite mortality %
• GROUP 1	95
• GROUP 2	95
• GROUP 3	90

Damages or remarks	No damage was found in any colonies
Conclusions	HopGuard II had a high efficacy but, there was no difference between 1 or 2 treatments. HopGuard II had a significantly higher efficacy than the control (Apivar).
Final comment	Excellent results from spring and autumn treatment with HopGuard II

Study 7.

Bassi: Unpublished data

SITE	Villa Luisa, Gorizia, North West Italy
IN CHARGE OF EXPERIMENTS	Dr. P. Bassi
FORMULATIONS	HopGuard II plus HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips, hanging over frames 3/4 and 7/8, diagonally opposite each other
Number of colonies	8 per treatment, 8 control
Number of Treatments	3
GROUPS	
• GROUP 1	2 HopGuard II strips replaced after 14 days, removed 14 days later
• GROUP 2	2 HopGuard strips, replaced after 7 and 14 days, removed 7 days later
• GROUP 3	Untreated control: 2 blank HopGuard II strips (no active ingredients), replaced after 14 days, removed 14 days later
Period of treatment	Summer 2015
Length of treatment	28 days

CONTROL TREATMENT	Apivar (amitraz) and Apibioxal (oxalic acid)
Results	Mean efficacy %
• GROUP 1	74.14
• GROUP 2	62.56
• GROUP 3	6.26
Damages or remarks	No damage was found in any colonies
Conclusions	HopGuard II provided moderate mite control and HopGuard was eliminated from further investigation.
Final comment	The efficacy was lower compared to other studies. The dosage should be modified.

Study 8.

Reference: Nasr, et. al. HopGuard II Concentration, Application and Honey Residues Experiment, Summer 2015 – Not Published

SITE	Crop Diversification Centre North, Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Medhat Nasr
FORMULATIONS	HopGuard II
PRESENTATION	Corrugated cardboard strips (44.4 x 3.2 cm)
DOSAGE	HopGuard II - 21g Hop Beta Acid per strip - 23g Hop Beta Acid per strip - 25g Hop Beta Acid per strip
Number of colonies	6 per treatment, 6 control, 5 for HopGuard II 25g/strip applied once
Number of Treatment	10
GROUPS	
• GROUP 1	25g/strip applied once
• GROUP 2	23g/strip applied once
• GROUP 3	21g/strip applied once

- GROUP 4 Apivar applied at second treatment date
- GROUP 5 Apivar applied at first treatment date
- GROUP 6 25g/strip applied twice, 14 days apart
- GROUP 7 23g/strip applied twice, 14 days apart
- GROUP 8 21g/strip applied twice, 14 days apart
- GROUP 9 Apivar applied at first treatment date and taken out at second treatment date
- GROUP 10 Control

Period of treatment June 16 to 30, 2015; Finishing treatment taken out August 10, 2015

Length of treatment 28 days

CONTROL

TREATMENT Apivar Strips (1 strip per 5 frames of Bees)

Results Efficacy %

- GROUP 1 48.8
- GROUP 2 38.1
- GROUP 3 41.6
- GROUP 4 62.3
- GROUP 5 73.0
- GROUP 6 61.9
- GROUP 7 63.4

- GROUP 8 64.1
- GROUP 9 19.8
- GROUP 10 28.5

Damages or remarks	No damage was found in any colonies
Conclusions	2 applications of HopGuard II at any dose provided better mite control than a single application. There was no significant difference between the efficacy and the concentration of the HopGuard II strip.
Final comment	2 applications as opposed to one of HopGuard II were significantly more effective at controlling Varroa mite populations. However, the 2 applications were only moderately effective at controlling the Varroa mite population in the summer. The different concentrations showed no significant difference in efficacy.

Study 9.

Theophilidis & Papachristoforou: Unpublished data

SITE	Aristotle University of Thessaloniki, Greece
IN CHARGE OF EXPERIMENTS	Prof. G. Theophilidis/Dr A. Papachristoforou
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	1 or 2 strips, inserted into the middle (Group 1) or edges (Group 2) of the winter cluster
Number of colonies	19 broodless colonies
Number of Treatments	3
GROUPS	
• GROUP 1	1 HopGuard II strip, removed after 14 days
• GROUP 2	2 HopGuard II strips, removed after 14 days
• GROUP 3	Untreated control: 1 blank strip, removed after 14 days
Period of treatment	Winter 2015
Length of treatment	28 days

CONTROL TREATMENT	Two applications of oxalic acid with 14 day interval

Results	Mite mortality %
• GROUP 1	87.41
• GROUP 2	94.99
• GROUP 3	9.37

Damages or remarks	No damage was found in any colonies.
Conclusions	Both HopGuard II treatments had high efficacy however, there was no differences between the treatments.
Final comment	1 strip works as well as 2 strips per hive.

Study 10.

Bassi: Unpublished data

SITE	Villa Luisa, Gorizia, North East Italy
IN CHARGE OF EXPERIMENTS	Dr. P. Bassi
FORMULATIONS	HopGuard II plus HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips, inserted through colony cluster diagonally opposite each other.
Number of colonies	8 per treatment, 8 control
Number of Treatments	3
GROUPS	
• GROUP 1	2 HopGuard II strips replaced after 14 days with 1 strip, removed 14 days later
• GROUP 2	2 HopGuard strips, replaced after 7 and 14 days, removed 7 days later
• GROUP 3	Control- 2 blank HopGuard II strops (no active ingredient), replaced after 14 days, removed 14 days later
Period of treatment	Winter 2015/2016
Length of treatment	28 days

CONTROL TREATMENT	Apivar (amitraz) and Apibioxal (oxalic acid)

Results	Mean efficacy %
• GROUP 1	95.02
• GROUP 2	96.03
• GROUP 3	9.04

Damages or remarks	Treatment resulted in some adult bee loss in small, wintering colonies.
Conclusions	HopGuard II provided excellent Varroa control in the winter.
Final comment	HopGuard II has a high efficacy but the dosages should be changed.

Study 11.

Belletti: Unpublished data

SITE	Boscat, Gorizia, North East Italy
IN CHARGE OF EXPERIMENTS	Dr. PA Belletti
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	One strip, inserted through colony cluster and removed after 14 days.
Number of colonies	8 per treatment, 8 control
Number of Treatments	1
GROUPS	
• GROUP 1	1 HopGuard II strip during 14 days.
Period of treatment	Winter 2015/2016
Length of treatment	14 days
CONTROL TREATMENT	Apivar (amitraz) and Apibioxal (oxalic acid)
Results	Mean efficacy %

- GROUP 1 96.01

Damages or remarks	No damage was found in any colonies.
Conclusions	HopGuard II provided excellent Varroa control in the winter.
Final comment	HopGuard II has a high efficacy when adapted for winter conditions.

Study 12.

Reference: Nasr, et. al. HopGuard II Concentration, Application and Honey Residues Experiment, Spring 2016 – Not Published

SITE	Crop Diversification Centre North , Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Medhat Nasr
FORMULATIONS	Hop Beta Acid (HBA)- HopGuard II
PRESENTATION	Corrugated cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	- Recommended dose: 1 strip per 5 frames of bees - Double dose: 2 strips per 5 frames of bees - Single application: only applied once - Double application: applied twice, 10 days apart
Number of colonies	5 per treatment, 5 control
Number of Treatments	5
GROUPS	
• GROUP 1	HopGuard Recommended dose Double application
• GROUP 2	HopGuard Double dose Double application

• GROUP 3	HopGuard Recommended dose Single application
• GROUP 4	HopGuard Double dose Single application
• GROUP 5	Control (no treatment)
Period of treatment	May 6-July 14, 2016
Length of treatment	Single application- 10 days, Double application- 21 days
CONTROL TREATMENT	Apivar Strips (1 strip per 5 frames of Bees) for 42 days

Results	Efficacy %
• GROUP 1	62
• GROUP 2	65
• GROUP 3	29
• GROUP 4	37
• GROUP 5	~20

Damages or remarks	No damage was found in any colonies
Conclusions	An early spring resulted in brood begin present during the experiment. Single applications of HopGuard II were ineffective at controlling Varroa. A double application was more effective, though there was no difference between using the recommended dose or the double dose.

Final comment	HopGuard II is not an effective mite control in the presence of brood. Two applications are necessary to control mites.

Study 13.

Vidondo: Unpublished data

SITE	Glaban Apiary, Tandil, Buenos Aires, Argentina
IN CHARGE OF EXPERIMENTS	Patricio Vidondo
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips hanging over frames 3 and 7
Number of colonies	16
Number of Treatments	3
GROUPS	
• GROUP 1	2 HopGuard II strips, replaced on day 15
• GROUP 2	2 HopGuard II strips, replaced on day 21
• GROUP 3	2 HopGuard II strips, replaced on day 28
Period of treatment	28th March – 6th June 2016
Length of treatment	56 days
CONTROL TREATMENT	Amivar 500 (Amitraz), 14 day treatment

Results	Mite mortality %
• GROUP 1	92.4
• GROUP 2	92.2
• GROUP 3	98.0

Damages or remarks	No damage was found in any colonies
Conclusions	The application of a second dose is needed and should be done on day 28.
Final comment	The results support the use of HopGuard for Varroa control.

3. Non-Safety Adverse Effects *(DACO 10.3; M10.3)*

This portion of the value package presents all information related to non-safety adverse effects (e.g., phytotoxicity to the host or rotational crop, damage to the site of application, etc.). This may include experimental results from research trials, published scientific literature, scientific rationales, and use history information.

3.1. General Factors Influencing Non-Safety Adverse Effects

Describe any general factors that may influence non-safety adverse effects, such as:

- environmental and edaphic conditions
- application timing relative to crop or pest growth stage
- description of other pest management strategies used during the study that could be relevant to potential adverse effects

N/A

Summary Studies done during early stages USA Hop Beta Acid (HopGuard) on bee toxicity. See Section 2.4 for additional studies.

Study 1.

Theophilidis & Papachristoforou: Unpublished data

SITE	Aristotle University of Thessaloniki, Greece
IN CHARGE OF EXPERIMENTS	George Theophilidis and Dr. Alex Papachristoforou
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips, hanging over brood frames 3/4 and 7/8, diagonally opposite each other and double dose, 4 strips positioned similarly.
Number of colonies	6 replicates per treatment group, 6 controls (18 total)
Number of Treatments	3
GROUPS	
• GROUP 1	2 HopGuard II strips, replaced after 14 days then removed 14 days later
• GROUP 2	4 HopGuard II strips, replaced after 14 days then removed 14 days later

• GROUP 3	Control: 2 blank HopGuard II strips (no active ingredient), replaced after 14 days then 14 days later
Period of treatment	Summer 2015
Length of treatment	28 days
CONTROL TREATMENT	Not required
Results	Overdosing (Group 2) resulted in higher initial bee losses for the first 48 hours at which time the effect subsided. No difference in bee mortality or bee behaviour was observed between Group 1 and Control.
Damages or remarks	With 4 strips per hive, the bees were subjected to excessive wetting which can cause bee mortality. 2 queens were lost in overdosed colonies. No other behavioural aberrations were observed.
Conclusions	Bee mortality with the normal hive treatment (2 strips per hive) was not significantly different to Control. HopGuard II was generally well- tolerated, even at double the recommended dose but within the first 48 hours post application, more adult bees died using the higher dose than Control. After 48 hours, no further disturbance was evident in the overdosed hives or in any other hive.
Final comment	Two HopGuard strips per hive application are recommended.

3.2. Supporting information from earlier formulations of the product or similar Products

If information is available for product formulations tested during earlier stages of development, or similar products, rationales and bridging data should be presented in this section to demonstrate equivalence between the products.

Experimental data from trials that evaluated both formulations in the same trial should be presented in this section using a format similar to that presented above in Section 2.4 Support for Proposed Claims.

To avoid duplication, a reference to Section 2.2 may be presented here, and all information concerning previous or similar formulations as it relates to both efficacy and non-safety adverse effects can be presented in Section 2.2.

Summary Studies done during early stages USA Hop Beta Acid (HopGuard) on bee toxicity. See Section 2.2 for additional studies.

Study 1.

Rademacher: Unpublished data

SITE	Free University of Berlin, Germany
IN CHARGE OF EXPERIMENTS	Dr. Eva Rademacher

FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips hanging over the frames into the winter cluster
Number of colonies	18, derived from nuclei colonies
Number of Treatments	2
GROUPS	
• GROUP 1	2 strips each
• GROUP 2	Untreated control (8 colonies)
Period of treatment	Winter 2010-11
Length of treatment	17 days
CONTROL TREATMENT	Oxuvar

Results	Bee mortality %
• GROUP 1	7.2
• GROUP 2	0.7

Damages or remarks	Mite mortality was significantly higher in treated colonies than in the untreated control.

Conclusions	The 17 day treatment with HopGuard® strips was generally well tolerated by the bees. However, bee mortality was significantly higher
Final comment	Hopguard II could be a potential product for Varroa control.

Study 2.

Rademacher: Unpublished data

SITE	Free University of Berlin, Germany
IN CHARGE OF EXPERIMENTS	Dr. Eva Rademacher
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	One strip per colony with a mean dosage of 260 µg per bee
Number of colonies	3
Number of Treatments	2
GROUPS	
• GROUP 1	HopGuard® strip placed on neighbouring comb to brood comb
• GROUP 2	Untreated control
Period of treatment	June 6-24, 2011
Length of treatment	18 days
CONTROL TREATMENT	Oxuvar

Results	Brood mortality %
• GROUP 1a	39.6
• GROUP 1b	6.5
• GROUP 2	17.7

Damages or remarks	The first five days of a treatment with HopGuard can cause a significant reduction in the number of brood in cells near the treatment strip. The brood combs away from the treatment strip showed brood mortality compared to the rest of the colony.
Conclusions	For a treatment during brood rearing, the HopGuard strip should be placed on brood free comb with one comb in between treatment and brood comb.
Final comment	HopGuard strips should be placed on brood free comb.

Study 3.

Rademacher: Unpublished data

SITE	Free University of Berlin, Germany
IN CHARGE OF EXPERIMENTS	Dr. Eva Rademacher
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	Portion of HopGuard® strip pinned on to a piece of comb
Number of colonies	2 x 3 small cages with 50 bees each per concentration, 1500 bees total
Number of Treatments	5
GROUPS	
• GROUP 1	9.8 µg beta acids per bee (3.52 cm² strip per cage)
• GROUP 2	12.5 µg beta acids per bee (4.48 cm² strip per cage)
• GROUP 3	15.2 µg beta acids per bee (5.44 cm² strip per cage)
• GROUP 4	20.5 µg beta acids per bee (7.36 cm² strip per cage)
• GROUP 5	Untreated control
Period of treatment	September – November 2011
Length of treatment	Bee mortality was monitored for 72 hours after treatment

CONTROL	Portion of plain cardboard strip pinned on to a piece of comb
TREATMENT	
Results	Bee mortality %
• GROUP 1	1.0
• GROUP 2	2.0
• GROUP 3	1.0
• GROUP 5	0.4
Damages or remarks	The dermal application of individual trickling of HopGuard® on to the bees was not tolerated by the bees, probably caused by the moistening agent. The mortality was significantly increased compared to the control group. Therefore it was necessary to return to the posology of the final product for the dose-response-relationship test in the laboratory.
Conclusions	The treatment with HopGuard® strips in different dosage was well tolerated by the bees. The mortality was in the range of the corresponding control group, no significant differences occurred.
Final comment	It was not possible to establish a dose-response relationship for bee mortality using HopGuard® strips.

Study 4.

Ahumada-Segura: Unpublished data

SITE	USDA-ARS Carl Hayden Bee Research Centre Apiary, Tucson, AZ
IN CHARGE OF EXPERIMENTS	Fabiana Ahumada-Segura
FORMULATIONS	Hop Beta Acid (HBA)
PRESENTATION	Cardboard strips (44.4 x 3.2 cm)
DOSAGE	2 strips per hive
Number of colonies	10 treatment, 10 control (20 total)
Number of Treatments	2
GROUPS	
• GROUP 1	Untreated control
• GROUP 2	3.84 g of a 16% HBA solution on cardboard strips
Period of treatment	
Length of treatment	3 days
CONTROL	
TREATMENT	

Results	Average number of dead bees before/after treatment
• GROUP 1	N/A- not significantly different
• GROUP 2	6.9/8.2
Damages or remarks	No damage was found in any colonies
Conclusions	No significant increase in bee mortality during sampling period.
Final comment	

3.3. Support for Proposed Claims *(DACO 10.3.2; M10.3.1)*

3.3.1. Host Crop Claims

Each claim should be identified (i.e., each application timing, method of application, pest, tank mix, etc.), and the approach and information used to support each of these claims, with respect to non-safety adverse effects, should be clearly indicated.

Claims may be organized in subsections by host crop, application timing/method, etc., whichever is best suited to the specific claims being proposed. A description of the effects of the treatment on the crop or use site and whether the effects were permanent or temporary, should be included.

Each subsection should present all the various types of information available (i.e., experimental results from research trials, published scientific literature, scientific rationales, and use history information) to support the claims within the subsection. It is important to note that this section of the value summary consists of a synopsis of all supporting information on non-safety adverse effects and not a compilation of the individual reports. Copies of individual reports should be submitted as a separate component of the value information package.

When crop tolerance or use site research trials are presented, a summary of the host/site reactions to the treatment as they relate to the use pattern employed in the studies should be included. Any differences between the tested use pattern and the proposed use pattern should be explained. Individual trial reports should be included in the value package.

When published scientific literature is presented, an explanation of the relevance of the results to the proposed use should be provided. A comparison of the proposed use pattern and the use pattern employed in the studies should be included. All units should be expressed in metric to enable a direct comparison of the application rates. If the products tested in the study have equivalent products registered in Canada, they should be identified. Any unexpected results should be discussed. A copy of the published articles should be included in the value package.

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When use history information is presented, a summary of the information in the templates as it relates to non-safety adverse effects on the host or use site should be provided. This should include (1) a brief explanation of the similarities of the proposed use pattern and the registered use pattern in the foreign jurisdiction, (2) information on the crop/use site tolerance profile of the proposed use, and (3) validation information describing the source of the information and the extent of their direct experience with the product.

When scientific rationales are presented, a clear explanation of the basis for support of the proposed use should be provided. It should contain the appropriate level of detail to enable a conclusion regarding the proposed claims to be made.

Regardless of the type of information submitted, the applicant should provide an explanation if the use pattern in the supporting information submitted differs from the proposed use pattern.

The subsection should finish with the presentation of an overall conclusion made on the basis of an integration of all the types of information presented to support the claim.

3.3.2. Rotational Crop Claims *(DACO 10.3.3; M10.3.1)*

Each claim should be identified (i.e., each rotational crop and its proposed replanting interval relative to application), and the approach and information used to support each of these claims, with respect to non-safety adverse effects, should be clearly indicated.

N/A

4. Consideration of Benefits *(DACO 10.5; M10.4)*

Canada has approximately 9,800 beekeepers and 750,000 honey bee colonies in 2016. The value of Canada's honey industry has been estimated to be $231 and $158 million in 2015 and 2016, respectively (Statistics Canada, 2015). The value of honey bees to Canada's agricultural economy, including pollination of crops, high value cash crops and minor crops, is estimated to be over $2 billion.

The economic losses associated with Varroa mites are serious. In Canada the long term average colony mortality is reported to be approximately 15% (Pernal 2007). The colony mortality in Canada ranged from 30%-35% from 2007 to 2010. The range of colony mortality has decreased by 40-50% since 2011. The reduction in mortality is likely related to better control of Varroa mites using Apivar and the improvement of beekeeping management practices.

Based on the above information, Canadian beekeepers lost 600,000 honey bee colonies between 2007 and 2009, which is equivalent to 100% of the colonies in Canada mainly due to the failure of controlling Varroa mites. As a result, beekeepers have incurred additional costs for purchasing or making up replacement colonies and loss of crops. A study conducted by Alberta Agriculture and Rural Development in 2007 revealed that the economic loss experienced by the Alberta beekeeping industry was estimated to be between $16.7 and $24.6 million as a result of increased colony losses from 15% to 30% (Chaudhary and Nasr, 2007). A recent study in Alberta also showed that the potential economic losses could be as high as $70 million/year due to high prices of honey, bees and pollination services' fees. In Canada, the estimated economic impact of losing 30% of honey bee colonies in one year was calculated to be valued at approximately $200 million. The impact of lack of bees for crop pollination and yield would be very significant.

If Apivar fails, it will likely lead to high losses of bee colonies and a decreasing number of healthy honey bee colonies available for crop pollination. Indeed, more demand than supply was evident for pollination in British Columbia during the spring of 2008. Some blueberry pollination contracts could not be honoured and some Alberta beekeepers, who winter their bees in British Columbia, delayed moving their bees to help supplying bees to fill in these pollination contracts. In Alberta, beekeepers who have never moved bees for hybrid canola pollination rented their bees in 2008 to help meet the demand for canola pollination. Table 7 is a summary of the estimated economic impact on honey yield and pollination value when Varroa mites are not adequately controlled.

Consideration of the Pest Management Regulatory Agency (PMRA) to register HopGuard® II is very important to the Canadian beekeepers. Beekeepers will have access to a new and effective bee protection

tool. Varroa mites have not been exposed to this miticide. Consequently, Varroa mites don't have known resistance to this product. This product will fit in the Varroa integrated management system and provide another option to beekeepers. Thus, HopGuard® II will help to maintain healthy bees and a sustainable, competitive industry that can supply an adequate number of bee colonies for crop pollination.

4.1. Survey of Alternatives ***(DACO 10.5.1; M10.4.3)***

A list of conventional and non-conventional (e.g. biological pesticides) products registered in Canada for the same uses currently being proposed. If the current alternatives do not address grower/user needs, an explanation should be provided. The date when the search was conducted should be reported.

- **Varroa mites were first discovered in Canada in 1989. Honey bee colonies with this mite were destroyed in 1989 and 1990 because there were no registered chemical controls.**
- Apistan:
    - **In 1991, the Emergency Use Registration (EUR) of the synthetic pyrethroid acaricide, Apistan® (fluvalinate) to control the *Varroa* mite was granted. Apistan® was given full registration in 1993 (Clay 1996). It is believed that the enormous negative impact of Varroa mites on honey bees was averted in Canada because of early registration and adoption of an effective mite control product like Apistan®.**
    - **Until early 2000, the Varroa mite had been successfully controlled in Canada primarily using Apistan®. To a lesser extent some beekeepers were using formic acid,**

but with mixed results. In 2001, a population of Varroa mites resistant to the active ingredient fluvalinate (Apistan®) was discovered in Canada. Over the next four years, Varroa population testing for fluvalinate resistance (Pettis et al. 1998), showed that fluvalinate-resistant Varroa mites had become widespread across Canada. The development of strains of Varroa mites resistant to fluvalinate had also been documented in a number of countries including the United States.

- CheckMite+:
    - **In response to the impending threat that treatment-resistant Varroa mites posed on the Canadian beekeeping industry, in 2002 the Pest Management Regulatory Agency (PMRA) granted an Emergency Use Registration (EUR) of CheckMite+™ Beehive Pest Control Strip (10% coumaphos) for affected provinces. Similar to the fluvalinate-resistant Varroa mites, testing for coumaphos resistance showed that Varroa mites had developed resistance to coumaphos as early as 2007 in some Canadian provinces.**
    - **In 2008 Varroa mites resistant CheckMite+™ were reported in Canada.**
    - **In fact Varroa resistant to CheckMite+ and Apistan® were considered widespread. These two miticides currently don't address beekeepers' needs.**
- Apivar:
    - **In August 2008, at the request of the Canadian Honey Council and provincial governments, PMRA approved the EUR of Apivar® for the control of Varroa mites in honey bee colonies. In 2012, Apivar was granted conditional registration for use in Canada until December 31, 2018.**

- **Since then, beekeepers using Apivar® in spring or fall, have found it to have very good efficacy. Often the mite control is good enough that the colonies typically will need a single treatment during the bee season. In some cases, alternative mite control options have been used as a supplementary treatment when needed. This practice is used to slow down the development of resistance as part of a resistance development management strategy.**

- Thymovar:
  - **Is a registered product. It is an individual dose of Thymol in wafer. It requires ambient temperature (15-35°C)**
  - **The efficacy ranges between 74-95% when applied and temperature's requirements are met.**
  - **In Canada due to cold spring and variable temperatures during fall, the efficacy of the product is not predictable.**
  - **May reduce queen egg-laying activity; may increase adult and young larvae mortality; works best under warmer temperatures; may cause bees to beard in hot weather; human skin irritant.**
  - **It can be used as an option in an Integrated Pest Management Program. However, monitoring and repeat application might be needed.**

- Mite-Away Quick Strips® (MAQS®)
  - **MAQS®: saccharide gel strip in a laminated paper wrap formulation of 46.7% formic acid. It is recommended to use when outside day temperature 10-33ºC.**

- **In northern climates, the window of opportunity for effective formic acid treatment in the spring and fall has been extremely limited. MAQS™ has resolved a lot of the temperature dependency associated with using formic acid; however because of the colony size requirements for using MAQS™, it is estimated that this product may not work in up to 30% of colonies in some provinces at critical treatment periods in spring and fall.**

- **65% formic acid liquid**
    - In Canada 65% Formic acid liquid is permitted to be applied in soaked absorbing pads, slow release pads or Mitegone pads
    - Treatment options are Absorbing pad (Dri-Loc) (30-40 ml per 2 story hive) up to3 applications: one every 7-10 days; Slow release pad (250ml) once, Mitegone (120-125 g formic acid 65% per pad), one pad per 5 frames of bees; 2 times per year.
    - It is recommended to use when outside temperatures are between 10°C and 30°C, and hive entrances are fully open.
    - It has similar limitation as MAQS®

- **Oxalic acid**
    - Sugar syrup drip with a syringe or drenching applicator, also Sublimation.
    - It can be applied when populations increase in spring and populations decrease in fall when there is little brood and brood rearing is reduced.
    - Efficacy range is 82-99%, especially if there is no brood present in hives.

- Requires 2-3 applications.

4.2. Compatibility with Current Management Practices Including IPM *(DACO 10.5.2; M10.3.2, M10.3.2.1, M10.3.2.2, M10.4.4)*

A description of how the proposed use can be integrated into the production system, including its contribution to integrated pest management. For example, information regarding pesticide spray programs that incorporate the proposed use or operational trials in actual grower fields could be provided, when available. In addition, any recommendations by extension personnel or information demonstrating the product's role and contribution to sustainability, such as the use of the product in IPM programmes, should be included. Information on any potential adverse effects on beneficial insects, as it affects sustainability of the use, should be explained.

Varroa mites have developed resistance to most registered synthetic miticides i.e. Apistan and CheckMite+. The only synthetic miticide known to be effective is Apivar. This miticide has been used since 2008 by beekeepers in spring and fall to control mites. Although the efficacy of Apivar has been acceptable in Canada, beekeepers are worried about merging resistance at any time.

Canadian beekeepers also have access to registered organic acids and thymol based miticides (i.e. formic acid, oxalic acid and Thymovar). Formic acid has been available for beekeepers to use to control Varroa mites since the mid 1990's (Note to CAPCO C94-05) and subsequently Mite-AwayII™, MAQS, oxalic acid and Thymovar since the mid-2000's. Although formic acid, oxalic acid and thymol based treatments have been available for a number of years these products have never been well adopted by beekeepers. In addition to the user safety concerns regarding the use of strong acids in their hives, many beekeepers have been concerned with the negative impact the product could have on the bees if too much or too little of these products was released in the hive. Beekeepers have also been concerned with the variable levels of efficacy based on time of treatments, ambient temperature during treatment time and the presence of brood.

Imdorf, et. al. (1990) reported that optimum efficacy of formic acid based products is obtained when the ambient temperature is 18-25°C, with minimum night temperature no lower than 12°C during the application period. Ostermann and Currie (2004) reported greater consistency in formic acid evaporation is achieved when the ambient temperature outside the hive is greater than 15°C. Temperatures during formic acid application in bee colonies are critical for achieving adequate control of Varroa (Calderone and Nasr, 1999). Mite mortality was only 56% when the daily average released formic acid was below the recommended level (≥10 ml/day) during a fall treatment in upstate New York.

Given that oxalic acid should not be used when brood is present in the hive, it would be an understatement to say that this is a severe limitation for this product and that it cannot be relied upon to control a Varroa mite outbreak prior to the broodless period (i.e. late fall, winter or early spring). When oxalic acid is applied to colonies that contain brood, the efficacy is reduced by 25% (Marcangeli and Garcia, 2004). Therefore, beekeepers need to treat colonies during the broodless period in late fall/winter to achieve high efficacy using oxalic acid.

Thymovar is a thymol based product. It volatilizes and accumulates in bee hives when the ambient temperature is between 15-20°C. Once the thymol vapour is at a high enough concentration, it is more toxic to mites, but is not harmful bees. When the ambient temperature is over 20°C, the thymol vapour drives the bees away from the brood cluster. Therefore, the queen would stop egg- laying and the brood production can be reduced by up to 25%. This reduction in brood production will consequently reduce the number of bees in bee colonies and decrease the honey production.

4.3. Resistance Management *(DACO 10.5.3; M10.4.4)*

A description of how the proposed use contributes to resistance management, in consideration of other registered alternatives. Information on the following should be provided: resistance risk of the pesticide active ingredient, reports of resistance in the target pest, effectiveness of the product on pests, whether the product represents a new pesticide mode of action for the crop, and whether the product can be incorporated in a resistance management strategy as a tank mix partner or a rotational product. In addition, any reports on baseline sensitivity and resistance monitoring should be provided, if they are available.

Based on the mode of action of Potassium Salts of Hop Beta acids as a contact miticide that may cause damage to mites leading to killing mites, the development of resistance to HopGuard II is unlikely in Varroa mite populations.

4.4. Contribution to Risk Reduction *(DACO 10.5.4; M10.4.4)*

A description of how the proposed use contributes to risk reduction, in consideration of other registered alternatives. Discuss how the use of the product contributes to risk reduction. For example, use of the product may reduce reliance on chemical alternatives. If the proposed use is considered a replacement for a use that is being phased out through re-evaluation, it should also be stated in this section.

Canada has approximately 9000 beekeepers and 725,000 honey bee colonies. The value of Canada's honey industry is estimated to be worth approximately $157-$210 million (Statistics Canada, 2016). The value of honey bees to Canada's agricultural economy, including crop pollination, is estimated to be over $2 billion. The long term average colony mortality in Canada has been approximately 15%. When the Varroa treatment

failed in 2007-2010, honey bee colony mortality averaged 30% (CAPA National Survey, 2016). Investigating the possible causes of the failure to control mites showed that Varroa had developed resistance to CheckMite+. Thus, the PMRA actively responded to beekeepers' demands and registered Apivar to control Apistan and CheckMite+ resistant strains of Varroa mites in 2008. Since then, beekeepers have relied on Apivar for Varroa treatment. Consequently, the bee colony mortality decreased by 50% since 2011.

Based on the above information, Canadian beekeepers have lost 600,000 honey bee colonies from 2007 to 2011, which is equivalent to 83% of the colonies currently in Canada. Once beekeepers were able to successfully controlled Varroa mites, bee mortality was reduced to about 20%. This reduction in winter loss can likely be attributed to better control of resistant Varroa mites by using Apivar.

In addition to the potential loss of production from these dead colonies, beekeepers have also incurred additional costs for purchasing or making up replacement colonies. Beekeepers who divide their colonies to make new colonies to replace dead ones end up weakening their honey producing colonies. Consequently, honey production can be greatly affected. A study conducted by Alberta Agriculture and Rural Development in 2007 revealed that the economic loss experienced by the Alberta beekeeping industry was estimated to be between $16.733 and $24.655 million. In Canada, the estimated economic impact of losing 30% of the honey bee colonies in one year was calculated to be valued at $30-45 million (Chaudhary and Nasr, 2007).

Successive annual colony losses by Canadian beekeepers at levels exceeding the long term average of 15% are unsustainable. This situation would likely decrease the number of healthy honey bee colonies available for crop pollination. Table 2 is a summary of the estimated economic impact on honey yield and pollination value when Varroa mites are not adequately controlled.

Table 2. Potential Economic Impact of not adequately controlling Varroa mite.

Region	**Canada**
Number of bee hives	725,000
Number of beekeepers	7000
Value of cash Farm receipts	$157 Million
Value of contribution to pollinated crops	$2 Billion
Average honey production/hive	133 lbs(61kg)
Expected yield without treatment	93 lbs (42kg)
Expected yield with treatment	100 %
Average winter mortality with effective treatment	18%
Average winter mortality without effective treatment or use of proposed HopGuard II registered product	30% = 217,500
Expected average winter mortality with treatment including using HopGuard II registration	18%
Estimated crops loss without using proposed HopGuard II registered product	30%
Estimate of Honey and Hive Products Value without using proposed HopGuard II registered product	$110 Million

Estimate of Honey and Hive Products Value losses without using proposed HopGuard II registered product	$47 Million
Estimate of Pollinated Crop Value without using proposed HopGuard II registered product	$1.4 Billion
Estimate of Pollinated Crop Value losses without using proposed HopGuard II registered product	$600 Million

HopGuard® II is a valuable tool for controlling Varroa mites in honey bee colonies. Data demonstrated that HopGuard® II can provide 75- 98% control of Varroa mites when two consecutive applications are applied, 10 days apart in spring or fall. High Varroa control can be achieved when HopGuard® II is used, when little or no brood is present in the hives (i.e. in the late fall to early spring). Moreover, HopGuard® II will eventually contribute to sustainability for Varroa control, as it is compatible with current control products. It will be an important tool for enhancing the sustainability of Varroa mite management in bee colonies, as resistance is unlikely to occur with this product. It will also improve the management program for pesticide resistance by adding an alternative mode of action to those currently used.

4.5. Social and Economic Impacts *(DACO 10.4; M10.4.2)*

This section should describe any social or economic impacts associated with the proposed use such as effects on the sustainability of the sector or trade implications (i.e. Maximum Residue Limit, or MRL, issues; impact on competitiveness of Canadian growers). Information explaining why the product is needed as well as how

and to what extent product registration would benefit Canadian users should be provided. Elements such as crop value (farm-gate, market value), acreage devoted to crop, influence of the pest on crop quality and marketability, additional costs associated with the pest presence (e.g. drying costs for grain), indirect effects of the pest on the crop (e.g. alternate host for a crop disease), or priority status of proposed use according to the Grower Priority database, etc. could be considered. It would be useful to indicate if the use is an identified grower priority or whether the registration of the product would result in harmonization with US.

Information on indirect benefits that could result from the proposed use could also be included. An example is a use that could reduce overall fuel costs or reduce soil compaction. Additional information such as an attribute that contribute to a product's value could be included. For example, if a product is stable for longer periods without the need for refrigeration, this attribute could impact product cost.

Honey bees play an important role in the environment and agriculture. Every third bite of food is the product of honey bee pollination. In recent years when the honey bee colony mortality doubled due to what is known as Colony Collapse Disorder and failure of Varroa controls, the public showed concerns due to lack of honey bees for crop pollination which in turn could threaten our food supply.

In order to address bee health, beekeepers need to apply miticides at the recommended dosage to control Varroa in bee colonies. The application of these miticides is proven to be generally profitable in beekeeping. Their use has improved bee health and reduced bee colony mortalities. For example when mites developed resistance to Apistan and Checkmite+, the bee colony mortality doubled (30% per year) between 2007 and 2011. Once beekeepers used Apivar to treat resistant strains of Varroa mites, the annual mortality was reduced to 15-18%, which is acceptable long-term mortality.

Overall, beekeepers have been able to reduce the cost of production by reducing the cost of replacing dead colonies and increasing their honey production. This is mainly because they have been able to effectively control Varroa mites. This reinforces the view that using effective alternative means for Varroa control in an integrated system is important in order to have an environmentally sound system for beekeeping.

4.6. Health, Safety and Environmental Benefits *(DACO 10.5.5; M10.4.4)*

This section is a summary of any potential health, safety or environmental benefits that could result from the proposed use of the pesticide. It is not a summary of the information provided to support the human health or environmental risk assessment. Where applicable, information on the benefits of the proposed use should be provided. For example, the applicant could indicate that the proposed use seeks to control a poisonous plant, a plant disease with harmful effects on humans/livestock (e.g., ergot) or control an invasive species. A product with a higher potential for crop safety (lower phytotoxicity) or a broader spectrum of activity on pests would be beneficial. There could also be references indicating if the product could replace, or reduce applications of, chemistries with health or environmental concerns.

Because there is a possibility of accidental dermal exposure by workers, individuals should observe all precautionary and first aid statements on the product label. The label-required wearing of personal protective equipment including goggles, face shield, or safety glasses by workers. Based on the limited use pattern to bee hives, bystanders are not expected to be exposed to Hops Beat acid salts.

No environmental risk-reduction measures are required. Standard label statements to protect the environment are required.

Environment benefits

- HopGuard® II seeks to preserve the health of honey bee colonies by controlling a destructive parasite, thereby preserving the ecological services that honey bees provide, namely: pollination which is fundamental to production of many food crops and reproduction of non-food native plants in our natural ecosystems.

- HopGuard® II will be an invaluable addition as a rotational product to mitigate the development of resistance in mite populations to currently registered chemical control options.

- Used and unused strips are non-hazardous to the environment and can be disposed or in landfill. Some other miticides cannot be disposed of this easily.

- Hopguard® II may serve to replace older chemistries such as Coumaphos which residues in hives have been shown to be detrimental to honey bee health.

- A thorough coverage of the absence of environmental effects by HopGuard® II, including extensive scientific references, is provided in our Section 3 EPA application (reference 1), Non-target Effects Waivers for the Technical Grade Active Ingredient Potassium salts of hop beta acids and the End Use

Product HopGuard® This document provides 7 waiver requests for the Technical Grade Active Ingredient, Potassium Salts of Hop Beta Acids, and for the End Use Product-HopGuard®.

Human Health and Safety

- **Beta acids are the active ingredient in HopGuard® II. They are non-toxic to humans. They are considered low risk-miticides (bio-pesticide) that can be safely used on crops.**

- HopGuard® II presents a reduced risk to handler safety compared to popular organic acid-based treatments in terms of inhalation risk, and HopGuard® II is not expected to affect nerve function.
- The active ingredient is naturally found in some foods. Residues in honey are less concerning that synthetic miticide alternatives.
- A thorough coverage of the absence of health and human safety effects by HopGuard® II, including extensive scientific references, is provided in our Section 3 EPA application (reference 2), Health Effects Waiver Rationale for the Technical Grade Active Ingredient, Potassium Salts of Hop Beta Acids and the End Use Product-HopGuard®. This document provides 13 waiver requests for the Technical Grade Active Ingredient, Potassium Salts of Hop Beta Acids, and 13 waiver requests for the End Use Product-HopGuard®.

Summary and Conclusions

Honey bees play an important role in agroecosystems because they pollinate one-third of agricultural crops. In Canada, beekeepers keep over 725,000 bee colonies. Their contributions as managed pollinators are indispensable. It is estimated that the value of bee pollination to the Canadian economy is over $2 billion per year. These honey bees currently experience a suite of health threats, including colony collapse disorder and the ectoparasitic mite, *Varroa destructor* (Anderson & Trueman, 2000). Beekeepers have been struggling to control Varroa mites. Monitoring and Integrated pest Management are necessary tactics to control Varroa. This includes the use of genetically tolerant Western honey bees and control methods such as essential oils, organic acids, and synthetic miticides.

In Canada, three synthetic miticides are currently registered for Varroa control. These miticides include Apistan, CheckMite, and Apivar. Despite the risks associated with using synthetic miticides, such as residues and developing resistance, they remain the only constant method of managing Varroa mites. Essential oil and organic acid based miticides are also used as control agents for Varroa. Although they are alternative tools for Varroa control, their efficacy is variable and depends on the ambient temperature.

In order to improve the Integrated Pest Management Strategy and manage resistance to used miticides for Varroa mite control, it is recommended to use miticides with different modes of action when necessary. It was recently discovered that a natural compound from the hop plant, *Humulus lupulus*, is shown to be effective against Varroa mites. Research in the USA, Canada, and other countries has shown hop beta acid salt can be used as a new mite control agent. Following several years of laboratory and colony testing, a formulation was refined and the final product, HopGuard II, based on hop beta acids, was registered as a Varroa treatment in the USA in 2015. The efficacy ranged from 80-95% based on the presence of brood in bee colonies. It is a contact miticide, but the mode of action is unknown. In evaluating the impacts of HopGuard II on bees, there

were no significant effects on brood production and queens. Based on studies in Saskatchewan and Alberta, Canada, two applications of HopGuard II (1 strip/5 frames covered with bees, 10 days apart) yielded a high efficacy of 95-98%, with no adverse effects on the honey bee colonies. Similar results were reported in South Texas, USA.

Overall, HopGuard II represents another option to add to the Varroa control tool box. Beekeepers can integrate this product into their spring and fall Varroa treatment of bee colonies. This product has a different mode of action and can be used for the management of Varroa mites that are resistant to synthetic products. Thus, beekeepers will be able to efficiently control Varroa mites and successfully reduce bee mortality.

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HOPGUARD® II

Acaricide Proposed Label for Canada

For use in bee hives to control Varroa mites (*Varroa destructor*) on honey bees

ACTIVE INGREDIENTS: BY WEIGHT

Potassium Salts of Hop Beta Acids:

…….16.0%

(Measured as Hop Beta Acids)

OTHER INGREDIENTS: 84.0%

TOTAL 100.0%

DANGER

KEEP OUT OF REACH OF CHILDREN

NET CONTENT: □ **12** HOPGUARD®II **strips per pouch**

□ **24** HOPGUARD®II **strips per pouch**

□ **48** HOPGUARD® II **strips per pouch**

□ **96** HOPGUARD®II **strips per pouch**

Each strip is folded in half and contains 4.0 grams of potassium salts of hop beta acids.

Manufactured by:

Mann Lake Ltd

501 1st South

Hackensack, Minnesota, 56452, USA

PRECAUTIONARY STATEMENTS

Hazards to Humans and Domestic Animals

Danger: Corrosive. Causes irreversible eye damage. Do not get in eyes or on clothing. Wear (specify appropriate protective eyewear such as goggles, face shield, or safety glasses). Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse.

FIRST AID

IF IN EYES: Hold eyelids open and flush with a steady, gentle stream of water for 15- 20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for advice

IF ON SKIN OR CLOTHING: Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control center or doctor for further treatment advice.

IF SWALLOWED: Call a poison control center or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by poison control center or doctor. Do not give anything by mouth to an unconscious person.

Note to Physician: "Probable mucosal damage may contraindicate the use of gastric lavage".

PERSONAL PROTECTION EQUIPMENT (PPE): Applicators and other handlers must wear appropriate protective eyewear, such as face shield or goggles long sleeved shirt and long pants, waterproof gloves and shoes plus socks.

USER SAFETY RECOMMENDATIONS:

Users must:

- Wear chemical resistant gloves (e.g. nitrile) when handling the strips.
- Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.
- Remove clothing immediately if pesticide soaks through. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

DIRECTIONS FOR USE

For Varroa mite control in bee colonies: Strips must be applied at the rate of one strip per five standard Langstroth combs covered with bees in each brood chamber (For example two strips per ten combs covered with bees in each brood chamber).

No. combs covered with bees	≤ 5	6-10	11-15	≥ 16
No. of HopGuard strips	1	2	3	4

Strips are only placed in the brood chamber (not in the honey super). Folded strips must be opened and hung over one of the center brood combs near the middle of the comb with one-half of the strip on each side of the comb. If using a second strip, apply the strips in the brood chamber in the center of the cluster, with a minimum distance of 2 combs between the strips. Suspend the strips in the brood chamber in a way that the bees can walk on both sides of the strips. Strips are to be placed only in the brood chamber (not in the honey super). Do

not lay the strips on top of the combs. Leave strips in the colony for 10-15 day interval. Repeat as necessary, up to 2 applications when bee population increasing in spring and population decreasing late summer and fall.

A maximum of four applications per year. This limit includes all applications to a bee colony (usually during spring, summer, and fall). Application of strips should be based on levels of Varroa mite observed in the colony. Users must not harvest honey and wax from the brood chambers, only from the honey supers. For optimal results, apply HopGuard® II when little to no brood is present in the hive. Wear chemical resistant gloves (e.g. nitrile) when handling the strips. **DO NOT re-use the strips.**

Warning

Do not use excess HopGuard material left in the HopGuard® II pouch on bees. Adding HopGuard® II liquid directly on bees might cause excessive damage to bees, brood and queens.

RESISTANCE MANAGEMENT

Varroa mite populations can become resistant to pesticides. Resistance development is affected by both the frequency of application and rate/dose of application. After an application, the more susceptible pests die and the less susceptible ones survive, mate with other survivors, and reproduce. Most of the ensuing offspring inherit the parental resistance. Additional applications continue to kill only the remaining, susceptible individuals. Continued reliance on a single miticide class or miticide with the same mode of action will select for resistant individuals which will dominate the mite population in subsequent generations. In order to prevent resistance development and to maintain the usefulness of individual pesticides, the adoption of an appropriate resistance management strategy is vital. The Mode of Action (MOA) for hop beta acids is undefined at this time: however, it may cause death by penetration of the pest's thin exoskeleton and causing asphyxiation.

To delay resistance:

- When possible, rotate the use of miticides to reduce selection pressure as compared to repeatedly using the same product, mode of action or chemical class. If multiple applications are required, use a different mode of action each time before returning to a previously-used one.

- Base miticide use on Integrated Pest Management (IPM). This includes proper pest identification, monitoring for locality, specific economic threshold and economic injury levels, record keeping, and utilizing all available control practices (cultural, biological and chemical).

- Maximize efficacy by following all label instructions including dosage and timing of application.

- Continually monitor treated populations for development of miticide resistance and report suspected resistance to local extension specialists.

- Contact your local extension specialist for additional pesticide resistance/management recommendations, and/or IPM recommendations for your specific location.

- For further information or to report suspected resistance contact your local extension specialist.

- Remove strips if still in hive after 15 days.

RESTRICTIONS

- For in-hive use only.
- Maximum rate is one strip for every 5 combs covered with bees per application.
- Remove remaining strip(s) after 15 days from bee hives.
- Do not use HopGuard® II more than 4 times per year.
- Don't apply residual liquid in the package onto bees. Excess material may damage bees, brood and queens.

DISPOSAL AND DECONTAMINATION

Dispose of packaging and used strips in accordance with provincial requirements. For information on disposal of unused, unwanted product, contact the manufacturer or the provincial regulatory agency. Unused HopGuard® II strips, packaging material and residual liquid in the package are non-hazardous waste according to EPA and should be disposed of as non-hazardous solid waste per RCRA (i.e. place in garbage bag and dispose in landfill).

STORAGE AND SHELF LIFE

Unused strips should be stored in a tightly sealed, cool, dark area. When stored appropriately, this product should show no significant degradation if used before the expiration date.

NOTICE TO USER

This pest control product is to be used only in accordance with the directions on the label. It is an offence under the Pest Control Products Act to use this product in a way that is inconsistent with the directions on the label. The user assumes the risk to persons or property that arises from any such use of this product.

HOPGUARD® II

Étiquette d'un acaricide proposée pour le Canada

À utiliser dans les ruches pour lutter contre le varroa (*Varroa destructor*) présent sur les abeilles domestiques

MATIÈRES ACTIVES : EN POIDS

Sels de potassium d'acides bêta du houblon : ……. 16,0 %

(Mesurés sous la forme d'acides bêta du houblon)

AUTRES INGRÉDIENTS : .....84,0 %

TOTAL 100,0 %

DANGER

GARDER HORS DE LA PORTÉE DES ENFANTS

CONTENU NET : □ **12 languettes** HOPGUARD® II **par sachet**

□ **24 languettes** HOPGUARD® II **par sachet**

□ **48 languettes** HOPGUARD® II **par sachet**

□ **96 languettes** HOPGUARD® II **par sachet**

Chaque languette est pliée en deux et contient 4,0 grammes de sels de potassium d'acides bêta du houblon.

Produit fabriqué par :

Mann Lake Ltd

501 1st South

Hackensack, Minnesota, 56452, É.-U.

MISES EN GARDE

Risques pour les humains et les animaux domestiques

Danger : Corrosif. Cause des dommages irréversibles aux yeux. Éviter tout contact avec les yeux ou les vêtements. Porter (préciser la protection oculaire appropriée telle que des lunettes à coque, un écran facial ou des lunettes de sécurité). Se laver soigneusement les mains à l'eau et au savon après avoir manipuler le produit, avant de manger, de boire, de mâcher de la gomme, de fumer ou d'aller aux toilettes. Retirer et laver les vêtements contaminés avant de les réutiliser.

PREMIERS SOINS

EN CAS DE CONTACT AVEC LES YEUX : Maintenir les paupières écartées et rincer 15 à 20 minutes sous un filet d'eau continu. Le cas échéant, retirer les lentilles de contact après les 5 premières minutes et continuer de rincer. Appeler un centre antipoison ou consulter un médecin.

EN CAS DE CONTACT AVEC LA PEAU OU LES VÊTEMENTS : Retirer les vêtements contaminés. Rincer immédiatement la peau à grande eau pendant 15 à 20 minutes. Appeler un centre antipoison ou consulter un médecin pour obtenir des conseils sur le traitement.

EN CAS D'INGESTION : Appeler un centre antipoison ou consulter un médecin immédiatement pour obtenir des conseils sur le traitement. Faire boire lentement un verre d'eau à la victime si celle-ci est capable d'avaler. Ne pas faire vomir à moins d'avoir été avisé de procéder ainsi par le centre antipoison ou le médecin. Ne rien administrer par la bouche à une personne inconsciente.

Remarque à l'intention du médecin : « En raison des dommages probables aux muqueuses, le recours à un lavage gastrique pourrait être contre-indiqué. »

ÉQUIPEMENT DE PROTECTION INDIVIDUELLE (EPI) : Durant l'application ou toute autre manipulation, porter un équipement de protection oculaire tel qu'un écran facial ou des lunettes à coque, une blouse à manches longues et un pantalon long, des gants imperméables, des chaussures et des chaussettes.

RECOMMANDATIONS POUR LA SÉCURITÉ DE L'UTILISATEUR

Les utilisateurs doivent :

- porter des gants résistants aux produits chimiques (p. ex. en nitrile) pour manipuler les languettes;
- se laver les mains avant de manger, de boire, de mâcher de la gomme, de fumer ou d'aller à la toilette;
- enlever immédiatement tout vêtement imbibé de pesticide, puis se laver soigneusement et mettre des vêtements propres;
- retirer l'EPI tout de suite après avoir manipulé le produit; laver l'extérieur des gants avant de les enlever; se laver soigneusement dès que possible et enfiler des vêtements propres.

MODE D'EMPLOI

Traitement contre le varroa dans les colonies d'abeilles : Les languettes doivent être appliquées à raison d'une languette pour cinq cadres Langstroth normalisés couverts d'abeilles dans chaque chambre à couvain (par exemple, deux languettes pour dix cadres couverts d'abeilles dans chaque chambre à couvain).

N^{bre} de cadres couverts d'abeilles	≤ 5	6-10	11-15	≥ 16
N^{bre} de languettes HopGuard® II	1	2	3	4

Les languettes ne doivent être placées que dans les chambres à couvain (et non dans les hausses à miel). Les languettes doivent être dépliées et suspendues au-dessus d'une des chambres à couvain du centre, près du milieu du cadre, chaque moitié de la languette de part et d'autre du cadre. Si une deuxième languette est utilisée, la mettre dans la chambre à couvain, au centre de la grappe, en laissant au moins deux cadres de distance entre les languettes. Suspendre les languettes dans la chambre à couvain de manière à ce que les abeilles puissent se déplacer des deux côtés de la languette. Les languettes ne doivent être placées que dans les chambres à couvain (et non dans les hausses à miel). Ne pas poser les languettes sur le dessus des cadres. Les laisser 10 à 15 jours dans la colonie. Refaire, au besoin, jusqu'à deux applications lorsque la population d'abeilles s'accroît au printemps et qu'elle diminue à la fin de l'été et à l'automne.

Ne pas faire plus de quatre applications par année. Cette limite comprend toutes les applications faites à une colonie d'abeilles (généralement au printemps, à l'été et à l'automne). L'application des languettes devrait dépendre de la quantité de varroas observée dans la colonie. Les utilisateurs ne doivent pas recueillir le miel ni la cire des chambres à couvain, mais uniquement des hausses à miel. Pour obtenir des résultats optimaux, appliquer les languettes HopGuard® II lorsqu'il n'y a que peu ou pas de couvain dans la ruche. Porter des gants résistants aux produits chimiques (p. ex. en nitrile) pour manipuler les languettes. **NE PAS réutiliser les languettes.**

Avertissement

Ne pas verser le liquide excédentaire restant dans le sachet de HopGuard® II directement sur les abeilles, car cela pourrait causer des dommages excessifs aux abeilles, au couvain et aux reines.

LUTTE CONTRE LA RÉSISTANCE

Les populations de varroas peuvent devenir résistantes aux pesticides. L'acquisition de résistance dépend à la fois de la fréquence et du taux d'application ou de la dose appliquée. Après une application, les ravageurs les plus sensibles meurent et ceux qui le sont moins survivent, s'accouplent avec d'autres survivants et se reproduisent. La majorité des descendants qui s'ensuivent héritent de la résistance parentale. Les applications additionnelles continuent de ne tuer que les sujets sensibles restants. Le recours prolongé à une seule classe d'acaricides ayant le même mode d'action ou à un seul acaricide sélectionnera les sujets résistants, lesquels seront dominants dans les populations d'acariens des générations subséquentes. Pour prévenir l'acquisition de résistance et conserver l'utilité de chacun des pesticides, il est indispensable d'adopter une stratégie de lutte efficace contre la résistance. Le mode d'action des acides bêta du houblon n'a pas encore été défini; cependant, il peut entraîner la mort par pénétration du mince exosquelette du ravageur et causer l'asphyxie.

Pour retarder l'acquisition de résistance :

- Si possible, utiliser les acaricides en rotation, ce qui réduira la pression de sélection comparativement à l'utilisation répétée du même produit, de produits ayant le même mode d'action ou appartenant à la même classe chimique. Dans le cas d'applications multiples, utiliser chaque fois un produit ayant un mode d'action différent avant de revenir à un produit utilisé précédemment.

- Utiliser les acaricides en se basant sur les principes de la lutte antiparasitaire intégrée (LAI). Cela comprend l'identification adéquate du ravageur, la surveillance pour la localité, le seuil d'intervention économique particulier et les seuils de dommages économiques, la tenue des

dossiers et l'utilisation de toutes les pratiques de contrôle existantes (culturales, biologiques et chimiques).

- Maximiser l'efficacité en suivant strictement le mode d'emploi fourni sur l'étiquette, dont le dosage et le calendrier des applications.

- Exercer une surveillance continue des populations traitées pour vérifier si une résistance à l'acaricide est apparue et signaler toute résistance soupçonnée aux spécialistes locaux en vulgarisation.

- Communiquer avec votre spécialiste local en vulgarisation pour obtenir des recommandations additionnelles au sujet de la résistance aux pesticides et de la lutte contre cette résistance ou des recommandations sur la LAI pour votre région en particulier.

- Pour en savoir plus ou signaler une résistance soupçonnée, communiquer avec votre spécialiste local en vulgarisation.

- Retirer les languettes qui sont toujours dans la ruche après 15 jours.

RESTRICTIONS

- À utiliser uniquement dans la ruche.

- La quantité utilisée par application ne doit pas dépasser une languette par groupe de cinq cadres couverts d'abeilles.
- Enlever les languettes restantes dans les ruches après 15 jours.
- Ne pas utiliser HopGuard® II plus de quatre fois par année.
- Ne pas verser le liquide restant dans l'emballage sur les abeilles, car cela pourrait causer des dommages aux abeilles, au couvain et aux reines.

ÉLIMINATION ET DÉCONTAMINATION

Éliminer les emballages et les languettes utilisées conformément aux exigences provinciales. Pour tout renseignement concernant l'élimination des produits inutilisés ou superflus, s'adresser au fabricant ou à l'organisme de réglementation provincial compétent. Les languettes HopGuard® II inutilisées, le matériel d'emballage et le liquide restant dans l'emballage ne sont pas des déchets dangereux selon l'EPA; ils doivent par conséquent être éliminés comme des déchets solides non dangereux, conformément à la RCRA (c.-à-d. mis dans un sac à déchets et éliminés dans un site d'enfouissement).

ENTREPOSAGE ET DURÉE DE CONSERVATION

Les languettes inutilisées doivent être entreposées dans un contenant hermétique, au frais et à l'abri de la lumière. Lorsqu'il est entreposé comme il se doit et utilisé avant la date de péremption, ce produit ne se dégradera pas de manière importante.

AVIS À L'UTILISATEUR

Ce produit antiparasitaire doit être utilisé seulement selon le mode d'emploi fourni sur l'étiquette. Toute utilisation non conforme au mode d'emploi constitue une violation de la *Loi sur les produits antiparasitaires*. L'utilisateur assume les risques que l'emploi de ce produit comporte pour les personnes ou la propriété.

DACO 5.2

Report Title:

Use Description and Exposure Scenarios (Handler and Post-Application) for HOPGUARD® II Acaricide on Honey Bee Hives

Product: HopGuard® II

Active Ingredient: ***potassium salts of hop beta acids***

Authors: Jim Chaput, OMAFRA; Paul Kozak, OMAFRA; Jason Sproule, NSDA; and Medhat Nasr, AAF.

Date: January, 2017

Sponsor:

Agriculture and Agri-Food Canada Pest Management Centre

TABLE OF CONTENTS

Use Description and Exposure Scenarios (Handler and Post-Application) for HopGuard® II on Honey bee hives

SUMMARY

The following clothing and personal protective equipment is required during application and handling of the product:

- chemical resistant gloves;
- protective eyewear such as: face shield or goggles;
- long-sleeved shirt and long pants;
- shoes and socks;

An apiary is the location where hives are kept. A typical honey bee operation could contain approximately 40 hives on 1 acre of land. Apiary size will however, vary region to region. There are numerous beekeepers who keep only a few hives and instances where over a thousand hives may temporarily congregate to prepare for trucking to new locations. Given that treatment occurs to a small area within hives, there is no contact with the outside environment. For this reason, apiary size, and land area should have little impact to occupational exposure.

Assuming that it takes approximately 1-2 minutes for an applicator (typically only 1 person is needed) to treat one hive, the pesticide applicator may spend approximately 1 hour to treat the average apiary in its entirety. If visiting multiple apiaries over a typical 9 hour workday applicator exposure may be between 2.5 to 5 hours depending on hive and apiary configuration. 8 hours is an absolute worst case exposure scenario for applicators. While four (4) applications per year is the maximum allowed by proposed labeling, it is likely that three (3) applications in a year is a realistic scenario. If additional treatments are required, a rotation with an alternative miticide is likely. Thus the total exposure duration for HopGuard® II applications, based on a 10-15 day treatment period, to an average apiary is estimated at approximately 40-60 days per year. The potential for volatilization of active ingredient potassium salts of hop beta acids from the product packaging is insignificant.

Re-entry into treated hives is necessary to scout for pests and diseases, application of treatments for other pests and disease issues, provide protein supplement and sugar feeds, monitor queen health and replace queens as needed, harvest honey, or manage hive strength through splitting or addition of supers or exchange of frames of bees, brood or stored honey and pollen. In many apiaries, workers may enter every day to harvest honey, or to perform other operations. These activities involve minimal contact with treated portions of the hive. Most workers will use gloves for these tasks. A re-entry interval (REI) of 0 hours is proposed for honey bee hives. Specific REIs do not currently exist for other labeled varroa mite control products such as fluvalinate, formic acid or amitraz and given the reduced risk nature of HopGuard® II it is proposed that labeling remain consistent between products.

Based on the above information, use of HopGuard® II on Canadian apiaries under the proposed label requirements and current regulatory guidelines does not pose undue risk to workers applying the product or reentering treated hives to perform necessary work tasks.

INTRODUCTION

The purpose of this report is to fully describe the parameters and conditions for the use of HopGuard® II acaricide on honey bee hives and the human activity and exposure patterns associated with its use.

HANDLER (MIXER/LOADER/APPLICATOR) ACTIVITIES

Use Site Category

Livestock for food (USC 8)

Site of Application

HopGuard® II is intended to be used inside honey bee hives

Production System

The Langstroth hive design, which is the most common amongst North American beekeepers, is comprised of one or more vertically stacked wooden (sometimes stryofoam) boxes containing 8-10 removeable frames. Other designs that orient colonies in a more horizontal space are increasingly popular among small operations but still represent a very small fraction of the industry. Bees draw wax comb, a pattern of hexagonal cells, to fill the wooden and/or plastic frames. Lower boxes, known as brood chambers, are reserved for rearing brood. A single queen bee lays eggs in cells of the wax brood comb, and worker bees tend to the developing larvae and store pollen and nectar in the surrounding cells. Extraneous nectar is stored and ripened to honey in frames of honey comb located in the upper boxes (a.k.a supers) from which honey can be harvested for human consumption. Once honeycomb is full,

the bees will cap the open cells with wax at which point honey frames and supers may be removed for harvest. Honey is extruded from frames by gravitational or centrifugal force and filtered before sealing in jars, pails or barrels. In addition to honey production beekeepers may also manage their colonies for the production of additional honey bee colonies, or queen rearing. This involves removing frames of honey bee brood and live honey bees from one or more colony and assembling these to make up new colonies. In addition, many beekeepers are increasingly managing colonies for pollination services, whereby colonies are temporarily moved to a location adjacent to a flowering crop. In some cases, honey production may be secondary to pollination and production of additional honey bee colonies.

Nature of the Effects on Harmful Organisms

The active ingredient, potassium salts of hop beta acids works on contact with varroa mites (*Varroa destructor).* Honey bees disperse the active ingredient throughout the hive during the course of grooming and direct body-to-body contact. The specific toxicological mechanism(s) are not well understood but hop beta acids have been shown to repel feeding and oviposition as well as reduce survival of adult varroa mites in hives and other mite pest species such as the two-spotted spider mite (*Tetranychus urticae* Kock) on plants in crop systems.

Formulation

HopGuard® II is a corrugated cardboard, mylar-backed strip moistened with potassium salts of hop beta acids in propylene glycol solvent and an emulsifier (polysorbate).

Details of Intended Use, Rate of Use and Rate of Active Ingredient

Each HopGuard® II strip contains 4.0 grams of potassium salts of hop beta acids. For honey bee hives, the recommended rate is one strip per five standard Langstroth frames of comb covered with bees in

each brood chamber. In a single brood chamber hive the maximum number of strips would be 2, and 4 strips for a double brood chamber hive.

Size of Producing Areas

The following table shows the total size of the honey bee hive production system on which HopGuard® II is proposed for registration in Canada, as well as average size for a typical apiary.

Table 1: Honey bee hives in Canada

	Total Number of Hives in	**Smallest Number of Hives per**	**Largest Number of Hives per Apiary(2,3)**	**Average Number of Hives per Apiary(4)**
Honey bee hives	725,000	1	1,000	40

1. CAPA National Survey Committee & Provincial Apiculturists 2016. Canadian Association of Professional Apiculturists

   Statement on Honey Bee Wintering Losses in Canada.

2. An apiary may be defined as a location where any number of hives are kept.

3. In preparation for transport large numbers of hives may be temporarily gathered in marshalling yards and mite treatments may be applied in these locations.

4. The number of hives per apiary is dependent on nearby floral resources, time of year, management goals of the beekeeper and may vary substantially between regions.

Area Treated Per Work Day

Bee hives are typically restricted to small, defined areas. Although bees typically forage beyond the land occupied by the apiary (up to a 2.5 km radius from their hives), most apiaries are small, accommodating all hives on less than 1 acre of land. Beekeepers may operate any number of apiaries. Treatments for varroa are applied within the colony and there is no contact with the surrounding environment.

Treatment is recommended when mites exceed a treatment threshold which is based on mite population growth models and economic injury levels. Scouting may be based on a sample of hives within each apiary but treatment to all hives within the apiary operation will typically occur once the treatment threshold is exceeded. Such use practices are in accordance with Integrated Pest Management programs, as is the use of selective alternative miticides such as: Apivar®, Apistan®, Thymovar®, Oxalic acid and Formic acid.

Data concerning average numbers of hives per apiary in Canada is unavailable, and would likely be skewed by extremely small (one or two hive) operations, and may vary greatly by region. Instead, 40 hives could be considered a sustainable number of hives per apiary in most regions (Table 1). In observations of typical apiary operations application rates of 40 hives / hour are expected.

The typical workday for workers in a beekeeping operation is 8 to 9 hours. In a typical workday a worker may travel to multiple apiaries to treat hives as well as perform additional types of colony management. For medium to large size operations, applications may be the only activity performed on a given day and applicators work individually or in pairs. It is estimated that applicators may handle each strip for 20 seconds and each colony may receive between 1-4 strips. Given time spent travelling between apiaries and opening and closing of hives, the actual handling time

of strips is likely limited from 2.5 to 5.0 hr per applicator per day. In small operations, beekeepers may perform various other

apiary tasks within the same day, further limiting time spent handling miticide strips. Hives are not usually re-opened for at least a few (3) days.

Assuming, as a worst case scenario, that one person treats 40 double brood chamber hives/hour, approximately 1440 strips per day totaling 8 hours of handling time.

Method of Application and Type of Equipment Used

HopGuard® II strips come ready to use. Application of strips requires cutting packaging and simple placement of strips directly into the hives. No mixing of the product with water or other solvents is necessary. No loading of the product into a sprayer or other mechanical applicator occurs.

Maximum Number of Applications and Their Timing

The HopGuard® II label will allow a maximum of 4 applications per season per colony. Strips remain in hives for 10-15 days. Hives are retreated as necessary, up to 2 times when bee population is increasing in spring and 2 times as populations are decreasing late summer and fall. This limit includes all applications to a bee colony (usually during spring, summer, and fall).

Individuals Involved in Mixing/Loading and Applying the Product

In small to mid-size apiary operations, the beekeeper is the individual applying the product. In the largest operations, a supervisor, foreman or worker might perform the application. Though loading of mechanical applicators is not required, strips from multiple packages may be pre-loaded into plastic totes at the start of the day.

Application Procedure

HopGuard® II will be packaged in laminated foil and film. Users must cut the package to remove the miticide strips. Folded strips must be opened and hung over one of the center brood frames near the middle of the comb with one-half of the strip on each side of the comb. If using a second strip, both strips are applied in the brood chamber in the center of the cluster, with a minimum distance of 2 frames between the strips. Strips are suspended in the brood chamber in a way that the bees can walk on both sides of the strips ensuring full contact of bees with the surface of the strip. Strips are to be placed only in the brood chamber (not in the honey super). Strips are not to be laid along the top of the brood frames or bottom board, or any other manner than prescribed above. Strips are left in the colony for 10-15 days.

Personal Protective Equipment and Clothing

The following clothing and personal protective equipment are required by product labeling for handling of the product including application and clean-up:

- chemical resistant gloves;
- protective eyewear such as: face shield or goggles;
- long-sleeved shirt and long pants;
- boots and socks;

These requirements are listed on the label under USER SAFETY RECOMMENDATIONS. In addition beekeepers typically wear full sleeve bee suits or jackets and veil which prevents touching their face.

POST-APPLICATION ACTIVITIES

Summary of Post Application Exposure

Re-entry into treated honey bee hives is necessary to perform the following types of activities:

- Removing HopGuard® II strips after 10-15 days
- Scouting for insects and diseases
- Employing other IPM strategies
- Harvesting honey
- Managing colony size
- Providing protein and sugar feed supplements

For honey bee hives, workers may enter every day to harvest the honey or perform routine management. At a minimum hives should be inspected every three weeks to scout for health issues and perform basic management activities. Meanwhile, most other activities need to be performed through most of the life of the hives. Often hive activities can be performed without direct contact of the surfaces to which HopGuard® was applied.

Most workers use gloves for re-entry activities; either chemical resistant gloves for phytosanitary reasons or leather bee gloves for protection from bee stings. Workers typically wear a long-sleeved shirt and long pants as standard clothing in the apiary. A re-entry interval (REI) of 0 hours is proposed for HopGuard® II use on honey bee hives.

Re-entry Activities

IPM Scouting

Most apiaries employ integrated pest management (IPM) programs. At least bi-weekly, scouts enter the hives to evaluate disease pressure and pest threshold levels. The scout may contact the wax comb in brood chambers and honey supers when handling the hives for closer inspection or for sampling, but contact is generally intermittent or incidental. In most cases it is possible to avoid direct contact with comb on which the HopGuard® II strips are placed. Secondary contact may occur from contact with bees that have contacted strips. Indirect contact from handling of combs which bees have distributed HopGuard® II over is likely.

Applying other pesticides

Application of other pest/disease control products after HopGuard® II application, occurs prophylactically or if disease or pest pressures meet a threshold level. However, applicators are expected to have very minimal contact with surfaces previously treated with HopGuard® II because (1) application optimally does not require or result in significant contact, and (2) applicators wear personal protective equipment which, at minimum, includes gloves, full length pants and sleeved shirt or jacket and veiled face protection.

Harvesting honey

Harvesting honey or placement of empty honey supers on hives typically does not require examination of brood chambers where HopGuard® II strips are located. Exposure is therefore

considered to be minimal as (1) contact with treated surfaces need not occur and (2) harvesters wear either personal protective equipment which, at minimum, includes gloves, full length pants and sleeved shirt or jacket and veiled face protection.

Splitting hives

Colonies in hives may be divided to manage the size of existing colonies or create new colonies/hives. Splitting requires removal and handling of brood combs including treated surface. If the split is made during the 10-15 day application period, handling of the HopGuard® II strip may occur. Workers are expected to contact the strips in a manner and time period similar to initial application. However, total exposure over the course of the workday is expected to be much less than initial application as the procedure of splitting hives will take substantially more time than re- handling of strips. Workers wear personal protective equipment which, at minimum, includes gloves, full length pants and sleeved shirt or jacket and veiled face protection.

Removal of HopGuard® II strips

Following the 10-15 day treatment period workers may remove miticide strips. Much of the active ingredient is expected to have been dissipated throughout the hive by this time. Furthermore bees may have significantly degraded miticide strips through chewing of the cardboard carrier material. Workers are expected to wear personal protective equipment which, at minimum, includes gloves, full length pants and sleeved shirt or jacket and veiled face protection.

Other activities

Other activities such as re-queening hives, removal of queen cells, managing colony growth, providing protein or sugar feed supplements are carried out as needed. These activities do require entering brood chambers and

manipulation of brood frames. However, these activities are not anticipated to occur for a minimum of three days post application and contact is expected to be similar to IPM Scouting (see above).

Timing, Frequency and Duration of Re-entry Activities

A typical work day for beekeepers is 8 to 9 hours. Re-entry activities are constantly required in apiaries, thus workers often spend entire work days performing various re-entry activities. The following table is provided to represent what a maintenance program at typical apiaries might entail, in terms of frequency and duration of activities. It must be noted that because there is variability in apiary size within and between provinces, there is also variability in frequency and timing of the activities described.

Table 2: Frequency and Timing of Apiary Activities in Canada

System	Activity	Frequency	Timing*
Honey bee	Scouting Honey harvest	Every 2 - 3 weeks Weekly	4-1 to 10-1 5-3 to 9-4 3-2 to 5-2
	Providing Feed	Every 1 – 2 weeks	and 8-2 to 10-2
	Requeening	Once / year	As needed; 4-1 to 8-3
	Supering	2 weeks	As needed; 5-1 to 9-3
	Splitting	1-2 times per year	5-1 to 7-2

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hives

	Applying other pesticides	Up to 12 applications per year (some are concurrent)	3-2 to 11-1

* Indicates month and week as "[month number]-[week number]". E.g., 12-1 indicates December, week 1.

Workers Performing Re-entry Activities

Individuals conducting the re-entry activities listed above are males and females of age 18 and older. These people may be full-time or seasonal contract workers. Since apiary operations are typically active throughout the spring, summer and early fall, seasonal employees (6-8 months per year) are most common. 1-2 fulltime workers may be employed year-round. Family members are also often involved in operation of keeping bees.

Principal Sources of Exposure

In re-entry activities, exposure will be primarily from hand and forearm contact with treated combs in the brood chamber. Exposure is minimized by wearing of gloves and full sleeve clothing.

Personal Protective Equipment and Clothing

Most workers use leather bee gloves or chemical-resistant gloves for re-entry activities; scouts wear chemical-resistant gloves for phytosanitary reasons. Honey harvesters wear gloves for food safety reasons. All workers wear a long-sleeved shirt and long pants as standard clothing. In addition to this, workers may also wear a bee suit over the other items of clothing.

Re-entry Intervals

A re-entry interval of 0 hours, as is typical for in-hive miticide treatments, is proposed for honey bee hives.

BYSTANDER EXPOSURE

The levels of exposure of bystanders present outside the treatment area will be less than that experienced by the applicator or a worker, which has been deemed acceptable level. Bystanders rarely visit apiaries and would be strictly prohibited from entering areas where applications are being made, and would not have any extensive contact with the hives. Inhalation exposure is minimal due to the physical properties of the compound and the fact that applications are made to a very small area and placed within a contained space (inside of the honey bee colony) separated from the outside environment. Apiaries will typically be vacated once applications are completed. Due to the potential for honey bees to sting, bystanders are not common near apiaries and are not likely to approach the honey bee colonies.

Value Assessment Template

Note to Applicant: This is the value assessment template that outlines the information required to support an application to register a pesticide or to add a proposed use to a currently registered product. It is intended to assist applicants in the preparation of the Value Summary upon which the value assessment is based. This template should be used in conjunction with relevant guidance documents (Regulatory Directive 2013-03; Regulatory Proposal 2010-07; Memorandum on Tank Mixes, etc.), summary tables for efficacy or crop tolerance trials, and use history information templates, as appropriate. Applicants are strongly encouraged to request a pre-submission consultation with PMRA early in the process in order to obtain advice relevant to the content, organization, and quality of the information package.

The Value Summary document that results from completing this template should be loaded under DACO 10.1. A reference to the DACO number that contains information relevant for each section of the template is included (in italics). Please note that DACOs for microbial pesticides are preceded by the letter "M".

Value Summary.1

1. Introduction

1.2. Product Description ***(DACO 10.2.1; M1.1, M1.2, M1.3)***

Provide a description of the formulated product. Include information related to the following points:

- Product Name
  - **HopGuard® II**
- Registration number (if currently registered)
  - **Not currently registered**
- Active ingredient(s)
  - **Potassium salts of Hop Beta Acids**
- Guarantee: By Weight
  - Potassium Salts of Hop Beta Acids……..……..……16.0%
  - OTHER INGREDIENTS: ……..…..…..…… 84.0%

  TOTAL 100.0%
- Formulation type
  - **IF = Impregnated Fabric (from SPSF 6003). HopGuard® Liquid (MA) is impregnated on corrugated cardboard-Mylar strips.**

Provide a description of the active ingredient(s). Include information related to the following points:

- Active ingredient

- **Potassium salts of Hop Beta Acids**

- Chemical class
    - **Weak organic acids**
- Mode of action
    - **Currently unknown.**
- Site of action classification
    - **See mode of action section**
- Mechanism of action
    - **See mode of action section**
- Mechanism of selectivity
    - **See mode of action section**
- Absorption / translocation in plants
    - **Not applicable. See EPA environmental waivers.**
- Factors influencing availability, mobility, degradation and persistence in the environment.
    - **Not applicable. See EPA environmental waivers.**

1.2. Use Pattern *(DACO 10.2.1; M1.2)*

1.2.1. Registered Use Pattern

- Unregistered product. There is no label in Canada for HopGuard® II

1.2.2. Proposed Use Pattern / Amendments to Registered Use Pattern *(DACO 10.2.1; M1.2)*

- For Varroa mite control in bee colonies: Strips must be applied at a rate of one strip per five standard Langstroth frames covered with bees in each brood chamber (For example, two strips per ten frames covered with bees in each brood chamber).

No. frames covered with bees	≤ 5	6-10	11-15	≥ 16
No. of HopGuard® II strips	1	2	3	4

- Strips are to only be placed in the brood chamber (not in the honey super). Folded strips must be opened and hung over one of the center brood frames near the middle of the frame with one-half of the strip on each side of the frame. If using a second strip, the strips must be placed in the cluster with a minimum distance of 2 frames between the applied strips in a single brood box. If more than 2 strips are applied, the additional strips should be applied in the second brood box in the same manner. The strips should be suspended in the brood chamber in a way that the bees can walk on both sides of the strips to be exposed to the material.
- DO NOT lay the strips on top of the frames. DO NOT pour excess liquid onto frames. DO NOT re-use the strips
- Leave strips in the colony for a 10-15 day interval.

- Repeat as necessary, up to 2 applications when the bee population is increasing in the spring and when the population is decreasing in the late summer and fall.
- A maximum of four applications per year. This limit includes all applications to a bee colony (usually during spring, summer, and fall).
- Application of strips should be based on levels of Varroa mites observed in the colony and the number of frames covered with bees (cluster size).
- Users must not harvest honey and wax from the brood chambers, only from the honey supers.
- For optimal results, apply HopGuard® II when little to no brood is present in the hive.

- crop(s)/site(s):
    - Honey bee hives

- pest claims with an indication of level of control (e.g., control, suppression) for each pest:
    - Varroa mites, one application could provide an average efficacy of 45%, two consecutive applications applied 10-15 days apart could provide an efficacy of 80-95%

- product proposed for use (formulation and guarantee)
    - Impregnated Fabric (from SPSF 6003). HopGuard® Liquid (MA) is impregnated on corrugated cardboard-Mylar strips.
    - Guarantee By Weight

- Potassium Salts of Hop Beta Acids……16.0%
- OTHER INGREDIENTS: ….……...……..…….. 84.0%

TOTAL 100.0%

- application rate
  - Strips must be applied at the rate of one strip per five standard Langstroth frames covered with bees in each brood chamber (For example, two strips per ten frames covered with bees in each brood chamber).

No. frames covered with	≤ 5	6-10	11-15	≥ 16
No. of HopGuard® II strips	1	2	3	4

- adjuvant or any other spray solution additive (if any) and its rate
  - N/A

- application method (spray, granular, in-furrow, etc.)
  - Strips are only placed in the brood chamber (not in the honey super). Folded strips must be opened and hung over one of the center brood frames near the middle of the frames with one-half of the strip on each side of the frame. If using a second strip, apply the strips in the brood chamber in the center of the cluster with a minimum distance of 2 frames between the strips. If two strips are applied, the strips must be placed in the cluster with a minimum distance of 2 frames between the applied strips in a single brood box. If more than 2 strips are applied, the additional strips should be applied in the second brood box in the same manner. Suspend the strips in the brood chamber in a way that the bees can walk on both sides of the strips. Do not

lay the strips on top of the frames. Do not pour excess liquid onto the frames. Leave strips in the colony for a 10-15 day interval.

- number of applications per season or crop cycle, and interval between applications
  - Up to 2 applications when the bee population is increasing in the spring and when the population is decreasing in the late summer and fall. Leave strips in the colony for a 10-15 day interval.
  - A maximum of four applications per year. This limit includes all applications to a bee colony (usually during spring, summer, and fall).

- application timing relative to crop growth stage
  - When the bee population is increasing in the spring
  - When the population is decreasing in the late summer and fall.

- application timing relative to pest growth stage
  - When the Varroa mite population reaches the economic threshold of 1% or greater based on a washed sample of 300 bees
  - When there is a daily mite drop of 10 mites/day on a sticky board.

- pre-harvest interval

- N/A

- spray volume and concentration (as appropriate)

  - N/A

- nozzle type and spray quality if relevant to the proposed use (e.g., flat fan nozzle producing a medium spray)

  - N/A

- for rotational cropping claims, the appropriate interval after which a rotational crop can be planted

  - N/A

- General use directions, such as conditions that warrant the use of the higher or lower application rate, or shorter or longer application interval.

- Application of strips should be based on levels of Varroa mite observed in the colony. If Varroa mite levels are less than 1%, application should be stopped. If Varroa mite levels after the first application are above 1%, then a second application should be applied in the brood chamber, not in honey supers during the honey flow.

The proposed label text can be included in this section. Alternatively, proposed label text as it relates to specific claims can be presented in Sections 2.4 and 3.4 immediately preceding the information submitted to support the claims.

- Proposed label is presented in section 2.4 and 3.4

1.3. Description of the Pest Problem *(DACO 10.2.2; M10.4.2)*

Provide a description of the pest(s) proposed to be added to the label, including:

- common and Latin binomial name(s)
    - Common Name: Varroa mite
    - Latin binomial name*: Varroa destructor* (Truman and Anderson, 2000)

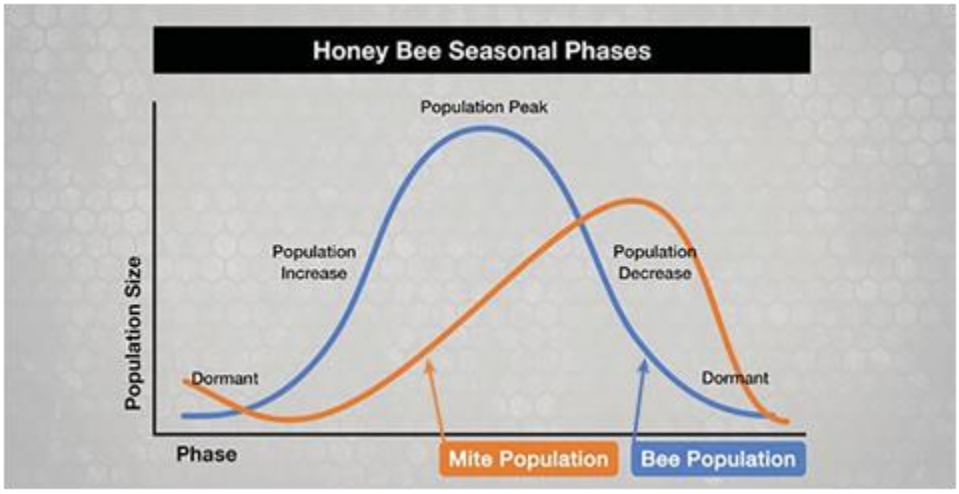
- the nature and severity of the damage to the crop(s) associated with the pest(s).
    - *Varroa destructor* is a natural parasite of the Asian honey bee, *Apis cerana*. These mites were accidently introduced into the Western honey bee, *Apis mellifera*. They were found in the USA

in the mid-1980's and in Canada in the early 1990's. They are external obligate parasites of worker and drone Western honey bees. They feed on the hemolymph of adult bees and developing pre-pupae and pupae. In this process, bee viruses such as the deformed wing virus (DWV), paralysis viruses and other viruses are transmitted to bees. Varroa also shortens the lifespan of honey bees and may even alter bee behaviour. A significant mite infestation will lead to the death of a honey bee colony within a year or two. Consequently, the *Varroa* mite is the parasite with the most pronounced economic impact on the beekeeping industry. It has also been considered a serious contributing factor to reported high colony mortality in Canada (Scott-Dupree 1996, Currie et al., 2010; Guzman et al., 2010, Hartman & Nasr, 2007, CAPA reports 2008-2016) and colony collapse disorder in the USA (Vanengelsdorp et. al. 2008).

- Relevant aspects of the pest(s) biology (e.g., life cycle) and its interaction with the crop should be included as appropriate. Complete descriptions of the pest species and their life cycles are not required.

    - Phoretic Varroa mites must enter bee brood cells before capping. In these cells, Varroa hides under the developing larvae that will become prepupae under the capping. The female mite that enters the cell is ready to reproduce. The female mite climbs onto the prepupae and begins feeding. Shortly thereafter, the mite lays its first egg on the surface of the cell wall. The egg is unfertilized and develops into a male mite. Subsequent fertilized eggs develop into female mites.

- During the Varroa feeding process on bee prepupae and pupae, mites transmit viruses. These viruses may cause damage to bees and one of the most telling signs of Varroa infestation is the occurrence of deformed wings caused by the deformed wing virus.
- Adult Varroa mites live up to 70 days. They reproduce on a 10-day cycle. Therefore, they have several reproduction cycles in their life. Overall, the Varroa population in a hive can multiply by about 12 times. High mite populations can cause crisis in the fall when summer bees and drones die, causing a quick crash and colony death in a short time (Fig. 1)



For details on Varroa and bee life cycle Check:

www.extension.org/pages/65450/varroa-mite-reproductive-biology

- If bee colonies are not treated in the spring, colony population build up will be compromised and honey yield will be reduced. Meanwhile, if bee colonies are not treated in the fall, winter bees will be impacted by Varroa infestation and high winter colony mortality is expected.
- During the active season, bees can drift to other adjacent colonies in an apiary, thereby, infecting the new colony. It is also known that bees from healthy colonies can rob honey from

failing colonies and bring Varroa to their own hives. These means of spreading Varroa are serious if mites are not controlled in infested bee colonies.

- Information on commercially acceptable levels of control, including economic thresholds, for each pest claim can be presented here.

  - The acceptable level of control is over 85%. It is advisable that a colony going to winter must have less than 1% Varroa mites using the wash method of 300 bees or less than 10 mites fallen on a sticky board in 24 hrs.

  - The economic threshold depends on the method of assessing Varroa mite infestation. The recommended methods for determining mite infestation include alcohol wash method and fallen mites on sticky boards.

  - The alcohol wash requires collecting 300 adult bees from bees on brood frames. The mites are separated from the bees using the Varroa mite shaker. If the number of collected mites is more than 7 mites/300 bees, then treatment is needed. This number is equivalent to a 3% infestation level as a correction factor of 1.9 mites/ sample added to the counted of fallen mites in the jar after shaking (Nasr et. al. 2017). For example if 7 mites fallen in the jar, total mites will be considered 9 mites. This will give 3% mite infestation in tested sample.

  - The sticky board method requires placing a sticky board in a modified bottom board to collect fallen mites. The installed sticky board is left for 3 days to collect fallen mites. All fallen mites are counted and the average fallen mites per day is calculated. The economic threshold for treatment using the sticky board is 10 mites/day.

  - Bee colonies must be sampled several times through the year to determine the infestation levels and detect mite population at critical times of the year in spring, late summer and fall.

Summary of Economic Thresholds.

Table 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 method) or natural *V. destructor* mortality per day.

Reference	Location, Time	Infestation (ether roll method)	Infestation (alcohol wash method)	Natural mortality (sticky board)
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%	
	Alberta, April		3%	>10 per day

2.	Nasr et al. 2008	Alberta, Fall	1%	10 per day

Product Efficacy ***(DACO 10.2, 10.2.3, 10.2.4; M10.1, M10.2.1, M10.2.2)***

This portion of the value summary presents all information related to efficacy. This may include experimental results from research trials, published scientific literature, scientific rationales, and use history information.

2.1. General Factors Affecting Product Efficacy

Describe any general factors that may influence product efficacy, such as:

- environmental and edaphic conditions
    - HopGuard® II is not affected by ambient temperature. It is unlike essential oils based products (i.e. Thymovar) and formic acid in that their efficacies depends on ambient temperature.

- application timing relative to crop or pest growth stage
    - HopGuard® II efficacy can be impacted by the presence of brood. If the product is applied in the spring and summer when too much brood is in the bee colonies, the efficacy decreases because mites confined in capped brood cells are not exposed to HopGuard® II.

- For high levels of mites and small population of bees: The activity of a small population of bees around the strips is very low. Therefore, the bees don't get enough material on their bodies to kill mites.
- During the honey flow when the bee population is too large (>50,000 bees/colony): The efficacy decreases due to low chances of bees contracting enough active ingredient from the HopGuard® II strips to kill mites on their bodies.

2.2. Supporting information from earlier formulations of the product or similar products

- HopGuard® was first developed as cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid. This formulation was registered in the USA.
- Research showed bees were able to chew the strips within a short period of time. Therefore, Impregnated Fabric (from SPSF 6003) was used for making new strips. HopGuard® Liquid (MA) is impregnated on these corrugated cardboard-Mylar strips.
- Guarantee: By Weight
    - Potassium Salts of Hop Beta Acids……..……16.0%
    - OTHER INGREDIENTS: ….……...…….....…. 84.0%

    TOTAL 100.0%

- Data supporting efficacies and bridging to the newly developed HopGuard® II are presented in this section.

Summary Studies done during early stages USA Hop Beta Acid (HopGuard) on efficacy and bee toxicity

Study 1.

Reference: DeGrandi-Hoffman, et. al. 2012. The effects of beta acids from hop (*Humulus lupulus*) on mortality of *Varroa destructor* (Acari: Varroidae). Exp. Appl. Acarol. 58:407-421.

SITE	USDA-ARS Carl Hayden Bee Research Centre Apiary, Tucson, AZ
IN CHARGE OF EXPERIMENTS	Fabiana Ahumada-Segura
FORMULATIONS	Hop Beta Acid (HBA)
PRESENTATION	Cardboard strips (44.4 x 3.2 cm)
DOSAGE	2 strips per hive
Number of colonies	7 per treatment, 7 control
Number of Treatments	3
GROUPS	
• GROUP 1	Untreated control
• GROUP 2	2 strips for 21 days
• GROUP 3	2 strips replaced with 2 new strips after 14 days

Period of treatment	September 2010
Length of treatment	21 days
CONTROL TREATMENT	Apistan applied after 21 days

Results	Total mite drop per colony	Efficacy (%)
• GROUP 1 (control)	436	N/A
• GROUP 2	582	88.2
• GROUP 3	1,017	81.3

Damages or remarks	No damage was found in any colonies
Conclusions	Average mite drop after 2 days did not differ between groups 2 and 3 but was significantly higher than control.
Final comment	By day 21, significantly more mites were counted in control colonies compared with groups 2 and 3.

Study 2.

Rademacher: Unpublished data

SITE	Free University of Berlin, Germany
IN CHARGE OF EXPERIMENTS	Dr. Eva Rademacher
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips inserted vertically into the winter cluster
Number of colonies	22
Number of Treatments	3
GROUPS	
• GROUP 1	HopGuard strips on top of broodless frames, repeated after 6 weeks
• GROUP 2	HopGuard strips in winter cluster
• GROUP 3	Untreated controls
Period of treatment	October (Pre-field) - December 2010 (Main Group)
Length of treatment	December 6-23, 2010 (Main Group)

CONTROL TREATMENT	Oxuvar treatment
Results	Mite mortality %
• GROUP 1	82.6
• GROUP 2	91.3
• GROUP 3	6.8
Damages or remarks	Bee mortality was significantly higher than the control (7.16%). It is assumed that chemical drippings caused the mortality. During the October treatment (pre-field test), there was no bee mortality observed.
Conclusions	Late autumn treatments resulted in high mite kill with a mean efficacy of >90%.
Final comment	HopGuard could be a good product for Varroa control.

Study 3.

Reference: Rademacher and Harz. 2011. Application study: hop acids to control varroosis - colony trials. Assoc. of Inst. for Bee Res., Report of the 58th Seminar in Berlin, 29-31 March 2011. Apidologie 42: 788-789

SITE	Free University, Berlin, Germany
IN CHARGE OF EXPERIMENTS	Eva Rademacher
FORMULATIONS	HopGuard strip
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips hanging over the frames into the winter cluster
Number of colonies	10 treatment, 8 control; derived from nucei colonies
Number of Treatments	2
GROUPS	
• GROUP 1	Control
• GROUP 2	HopGuard
Period of treatment	Winter 2010-11
Length of treatment	17 days

CONTROL TREATMENT	Oxuvar

Results	Mite mortality (%)
• GROUP 1 (control)	7.8
• GROUP 2	93.5

Damages or remarks	Treatment was well tolerated.
Conclusions	Efficacy was significantly higher than the control. The highest mite mortality of HopGuard occurred within the first 24 h.
Final comment	This study supports the further investigation and approval of HopGuard as a miticide in Europe.

Study 4.

DeGrandi Hoffmann: Population growth of Varroa destructor (Acari: Varroidae) in commercial honey bee colonies treated with beta plant acids

Gloria DeGrandi-Hoffman • Fabiana Ahumada • Robert Curry • Gene Probasco • Lloyd Schantz

Received: 3 September 2013 / Accepted: 28 April 2014

SITE	Adee Honey Farms, Fresno, California, USA
IN CHARGE OF EXPERIMENTS	Gloria DeGrandi Hoffmann
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips
DOSAGE	2 strips per hive
Number of colonies	48 split hives
Number of Treatments	4
GROUPS	
• GROUP 1	1 application, inserted April
• GROUP 2	1 application in April; 3 applications in September
• GROUP 3	3 applications, inserted September

• GROUP 4	Untreated control
Period of treatment	April-September 2011
Length of treatment	48 hours
CONTROL TREATMENT	Normal mite drop was observed in the control group.

Results	Mites per 100 bees in September (Average)
• GROUP 1	1.25
• GROUP 2	1.3
• GROUP 3	2.5
• GROUP 4	4.0

Damages or remarks	No damage observed in the treated colonies.
Conclusions	Colonies with 3 treatments in September had significantly fewer mites per 100 bees than colonies with 1 application or the untreated controls
Final comment	No efficacy data were reported.

Study 5.

DeGrandi Hoffmann: Population growth of Varroa destructor (Acari: Varroidae) in commercial honey bee colonies treated with beta plant acids

Gloria DeGrandi-Hoffman • Fabiana Ahumada • Robert Curry • Gene Probasco • Lloyd Schantz

Received: 3 September 2013 / Accepted: 28 April 2014

SITE	Pendell Apiaries Stonyford, California, USA
IN CHARGE OF EXPERIMENTS	Gloria DeGrandi Hoffmann
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips
DOSAGE	1 strip per package; 2 strips per hive
Number of colonies	22 packages; developed in Langstroth hives
Number of Treatments	5
GROUPS	
• GROUP 1	1 application, inserted June 22
• GROUP 2	3 applications, inserted June 22, August 4, and October 11
• GROUP 3	2 applications, inserted August 4 and October 11

• GROUP 4	Package treated control
• GROUP 5	Untreated control
Period of treatment	June-October 2011
Length of treatment	48 hours
CONTROL TREATMENT	Normal mite drop was observed in the control group.

Results	Mites per 100 bees in October (Average)
• GROUP 1	14.8
• GROUP 2	3.0
• GROUP 3	5.0
• GROUP 4	16.0
• GROUP 5	10.7

Damages or remarks	No damage observed in the treated colonies.
Conclusions	Group 2 and 3 had significantly lower number of mites per 100 bees than the untreated controls.
Final comment	No efficacy data were reported.

Study 6.

Reference*:* Vandervalk, et. al. 2014. New Miticides for Integrated Pest Management of *Varroa destructor* (Acari: Varroidae) in Honey Bee Colonies on the Canadian Prairies**.** J. Econom. Ent. 107(6):2030-2036.

SITE	Crop Diversification Centre North, Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Lynae Vandervalk
FORMULATIONS	Hop Beta Acid (HBA)/HopGuard
PRESENTATION	Cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	- 1 HopGuard strip applied for every 4-5 frames of bees in each brood chamber. - 1 Thymovar Wafer per colony, applied on the top of the brood chamber - Two 40 ml 65% Formic acid in Dri-Loc pads applied weekly for 3 weeks - 1 Apivar strip for every 4-5 frames of bees
Number of colonies	8 per treatment, 8 control
Number of Treatments	5

GROUPS

- GROUP 1 Apivar
- GROUP 2 Formic Acid
- GROUP 3 HopGuard
- GROUP 4 Thymovar
- GROUP 5 Control (no treatment)

Period of treatment September 5-October 18, 2011

Length of treatment 42 days

CONTROL TREATMENT Oxalic acid sublimation (2 g/hive)

Results

Table. 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	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).
Damages or remarks	No damage was found in any colonies as a result of HopGuard treatment
Conclusions	HopGuard provided 42.96% efficacy against Varroa mites. The HopGuard had significantly lower efficacy in comparison to Apivar, Thymovar, and Formic acid.
Final comment	HopGuard single application did not provide an adequate control in fall.

Study 7.

Reference: Rademacher, et. al. 2015. The development of HopGuard as a winter treatment against *Varroa destructor* in colonies of Apis mellifera. Apidologie 46:748-759

SITE	Birkenwerder, Germany
IN CHARGE OF EXPERIMENTS	Eva Rademacher
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard (16% w/w beta acids)
DOSAGE	2 strips for small colonies; 4 strips for large colonies
Number of colonies	30
Number of Treatments	4
GROUPS	
• GROUP 1	2 strips in small colonies
• GROUP 2	Small control colonies
• GROUP 3	4 strips in large colonies
• GROUP 4	Large control colonies
Period of treatment	November 2011

Length of treatment	14 days
CONTROL TREATMENT	30 ml Oxuvar

Results	Mite mortality %
• GROUP 1	83.8
• GROUP 2	94.2
• GROUP 3	83.0
• GROUP 4	83.8

Damages or remarks	No damage was found in any colonies.
Conclusions	The mean efficacies did not exceed 90% and were not significantly different than the controls treated with oxalic acid.
Final comment	HopGuard has moderate efficacy against *V. destructor*.

Study 8.

Reference: Rademacher, et. al. 2015. The development of HopGuard as a winter treatment against *Varroa destructor* in colonies of Apis mellifera. Apidologie 46:748-759

SITE	Celle, Germany
IN CHARGE OF EXPERIMENTS	Eva Rademacher
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard (16% w/w beta acids)
DOSAGE	2 strips for small colonies; 4 strips for large colonies
Number of colonies	30
Number of Treatments	4
GROUPS	
• GROUP 1	2 strips in small colonies
• GROUP 2	Small control colonies
• GROUP 3	4 strips in large colonies
• GROUP 4	Large control colonies
Period of treatment	November 2011

Length of treatment	14 days
CONTROL TREATMENT	30 ml Oxuvar

Results	Mite mortality %
• GROUP 1	75.3
• GROUP 2	93.4
• GROUP 3	88.2
• GROUP 4	96.1

Damages or remarks	No damage was found in any colonies.
Conclusions	The mean efficacies did not exceed 90% and were not significantly different than the controls treated with oxalic acid. The highest mite mortality occurred within the first 72 hours.
Final comment	HopGuard has moderate efficacy against *V. destructor.*

Study 9.

Reference: Vandervalk, et. al. 2014. New Miticides for Integrated Pest Management of *Varroa destructor* (Acari: Varroidae) in Honey Bee Colonies on the Canadian Prairies. J. Econom. Ent. 107(6):2030-2036.

SITE	Crop Diversification Centre North, Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Lynae Vandervalk
FORMULATIONS	Hop Beta Acid (HBA)/HopGuard
PRESENTATION	Cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	- 1 HopGuard strip applied for every 4-5 frames of bees in each brood chamber. - 1 Thymovar Wafer per colony, applied on the top of the brood chamber - Two 40 ml 65% Formic acid in Dri-Loc pads applied weekly for 3 weeks - 1 Apivar strip for every 4-5 frames of bees
Number of colonies	8 per treatment, 8 control
Number of Treatments	5
GROUPS	

- GROUP 1 Apivar
- GROUP 2 Formic Acid
- GROUP 3 HopGuard
- GROUP 4 Thymovar
- GROUP 5 Control (no treatment)

Period of treatment May 7 - June 18, 2012: Finishing treatment removed July 31, 2012

Length of treatment 42 days

CONTROL TREATMENT Apivar strips (1 strip per 5 frames covered with bees)

Results

Table. 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®	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).

Damages or remarks	No damage was found in any colonies as a result of HopGuard treatment but damaged was found as a result of Thymovar treatment
Conclusions	HopGuard provided 43.56% efficacy against Varroa mites despite it was applied 3 times, 10 days apart. The HopGuard had significantly lower efficacy in comparison to Apivar, Thymovar, and Formic acid. Bees were able to chew the strips within 5 days of treatments
Final comment	HopGuard application did not provide an adequate control in spring

Study 10.

Reference: Nasr, et. al. 2013. An effective improved application method of HopGuard for Varroa Control in Canada. Not published.

SITE	Crop Diversification Centre North, Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Medhat Nasr
FORMULATIONS	Hop Beta Acid (HBA)/HopGuard strips
PRESENTATION	Cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	- 15 g of liquid HopGuard in a Petri dish replaced every 10 days, 2 applications total - 15g of liquid HopGuard and 2.5 g of thymol powder in a Petri dish replaced every 10 days, 2 applications total - 2.5 g of thymol powder in a Petri dish replaced every 10 days, 2 applications total - 4 HopGuards strips* in a screened frame replaced every 5 days, 4 applications total - 4 HopGuard strips* that were incubated for 3 days at 25°C replaced every 5 days, 4 applications total

- 4 HopGuard strips* that were incubated for 5 days at 25°C replaced every 5 days, 4 applications total
- 4 HopGuard strips* replaced every 5 days, 4 applications total
- Control (no treatment)

*2 strips per box

Number of colonies 5 per treatment, 5 control

Number of Treatments 8

GROUPS

- GROUP 1 15g liquid HopGuard
- GROUP 2 15g liquid HopGuard + 2.5 g thymol powder
- GROUP 3 2.5 g thymol powder
- GROUP 4 Screened HopGuard
- GROUP 5 3 day incubated at 25°C HopGuard
- GROUP 6 5 day incubated at 25°C HopGuard
- GROUP 7 HopGuard
- GROUP 8 Control (no treatment)

Period of treatment August 7-September 3, 2013

Length of treatment	20 days
CONTROL	
TREATMENT	1 Apivar strip per 5 frames of bees for 42 days

Results	Average ± STD Efficacy (%)
• GROUP 1	10.8 ± 3.7
• GROUP 2	9.4 ± 2.2
• GROUP 3	42.2 ± 14.6
• GROUP 4	9.9 ± 2.0
• GROUP 5	11.3 ± 5.1
• GROUP 6	20.5 ± 4.2
• GROUP 7	56.7 ± 16.9
• GROUP 8	10.7 ± 4.0

Damage or Remarks	No damage was found in any colonies as a result of HopGuard treatment
Conclusions	High mite mortality was seen 1-3 days after application. The low efficacy for screened strips confirms that HopGuard is a contact miticide. Strips dried quickly and were then chewed by the bees.
Final comment	HopGuard did not adequately control Varroa, possibly due to the strip design.

2.3. Requirement for Adjuvants

If an adjuvant is proposed to be used with the product, present information in this section to demonstrate why the adjuvant is required, or under what circumstances it is required. This information may include experimental results from research trials, published scientific literature, scientific rationales, and use history information.

If more than one adjuvant is proposed to be used with the product, present information in this section to demonstrate the similarity or interchangeability of the adjuvants, when used in combination with the product.

Experimental results from trials that evaluated the product both with, and without the inclusion of an adjuvant in the same trial should be presented in this section using a format similar to that presented below in Section 2.4 Support for Proposed Claims.

- N/A

2.4 Support for Proposed Claims *(DACO 10.2, 10.2.3., 10.2.3.3, 10.2.3.4, 10.2.4; M10.1, M10.2.1, M10.2.2)*

Each claim should be identified (i.e., each application timing, method of application, pest, tank mix, etc.), and the approach and information used to support each of these claims, with respect to efficacy, should be clearly indicated.

Claims may be organized in subsections by pest, application timing/method, etc., whichever is best suited to the specific claims being proposed.

Each subsection should present all the various types of information available (i.e., experimental results from research trials, published scientific literature, scientific rationales, and use history information) to support the claims within the subsection. It is important to note that this section of the value summary consists of a synopsis of all supporting efficacy information and not a compilation of the individual reports. Copies of individual reports should be submitted as a separate component of the value information package.

When research trials are presented, a summary of the experimental results as they relate to the proposed claims should be included. The applicability of factors such as application rate and timing, application method, number of applications, application interval, etc., to the proposed use pattern should be explained. Inconsistencies between the test parameters and the proposed use relating to product formulation, location of the trials, assessment parameters, expected results, or other factors should be justified. Individual trial reports should be included in the value package.

When published scientific literature is presented, an explanation of the relevance of the scientific paper/s to the proposed use should be provided. A comparison of the proposed use pattern and the use pattern employed in the studies should be included. All units should be expressed in metric to enable a direct

comparison of the application rates. If the products tested in the study have equivalent products registered in Canada, they should be identified. Any unexpected results should be discussed. A copy of the published articles should be included in the value package.

When use history information is presented, a summary of the information provided in the templates should be presented. This should include (1) a brief explanation of the similarities of the proposed use pattern and the registered use pattern in the foreign jurisdiction, (2) information on the efficacy and crop tolerance profile of the proposed use, and (3) validation information describing the source of the information and the extent of their direct experience with the product.

When scientific rationales are presented, a clear explanation of the basis for support of the proposed use should be provided. It should contain the appropriate level of detail to enable a conclusion regarding the proposed claims to be made.

Regardless of the type of information submitted, the applicant should provide an explanation if the use pattern in the supporting information submitted differs from the proposed use pattern.

The subsection should finish with the presentation of an overall conclusion made on the basis of an integration of all the types of information presented to support the claim.

Study 1.

Reference: Nasr, et. al. 2013. An effective improved application method of HopGuard for Varroa Control in Canada. Not Published.

SITE	Crop Diversification Centre North, Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Medhat Nasr
FORMULATIONS	HopGuard and HopGuard II
PRESENTATION	- HopGuard: Cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid - HopGuard II: Corrugated cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	4 strips per hive for both HopGuard and HopGuard II
Number of colonies	6 per treatment, 6 control
Number of Treatment	5
GROUPS	
• GROUP 1	4 HopGuard strips replaced every 5 days, 3 times
• GROUP 2	4 HopGuard II strips applied once to colonies
• GROUP 3	4 HopGuard II strips applied twice replaced after 10 days

• GROUP 4	4 HopGuard II strips applied 3 times, replaced every 5 days
• GROUP 5	Control
Period of treatment	September 30 – October 21, 2013; Finishing treatment (Apivar) removed November 24, 2013
Length of treatment	21 days
CONTROL TREATMENT	Apivar Strips (1 strip per 5 frames of Bees)
Results	Alberta Efficacy %
• GROUP 1	90
• GROUP 2	73
• GROUP 3	96
• GROUP 4	98
• GROUP 5	18
Damages or remarks	No damage was found in any colonies
Conclusions	2 Applications of HopGuard II regardless of dose was very effective at controlling mites with an efficacy of 96% and 97% when applied twice and 98% when applied 3 times. There was no significant difference between 2 and 3 applications of HopGuard II and it efficacy as a miticide.

Final comment	2 applications of HopGuard II at the recommended dose of 1 strip per 5 frames of bees were effective at controlling mites in the fall.

Study 2.

Reference: Nasr, et. al. 2013. An effective improved application method of HopGuard for Varroa Control in Canada. Not Published.

SITE	Saskatchewan Ministry of Agriculture, Prince Albert, Saskatchewan, Canada
IN CHARGE OF EXPERIMENTS	Graham Parson and Geoff Wilson
FORMULATIONS	HopGuard I and HopGuard II
PRESENTATION	- HopGuard: Cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid - HopGuard II: Corrugated cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	4 strips per hive for both HopGuard and HopGuard II
Number of colonies	6 per treatment, 6 control
Number of Treatment	3
GROUPS	
• GROUP 1	4 HopGuard strips replaced every 5 days, 3 times
• GROUP 2	4 HopGuard II strips applied once to colonies

• GROUP 3	4 HopGuard II strips applied twice replaced after 10 days
Period of treatment	October 3-24, 2013
Length of treatment	21 days
CONTROL TREATMENT	Apivar Strips (1 strip per 5 frames of Bees)

Results	Saskatchewan Efficacy %
• GROUP 1	92
• GROUP 2	92
• GROUP 3	97

Damages or remarks	No damage was found in any colonies
Conclusions	2 Applications of HopGuard II regardless of dose was very effective at controlling mites with an efficacy of 96% and 97% when applied twice and 98% when applied 3 times. There was no significant difference between 2 and 3 applications of HopGuard II and it efficacy as a miticide.
Final comment	2 applications of HopGuard II at the recommended dose of 1 strip per 5 frames of bees were effective at controlling mites in the fall.

Study 3.

Ahumada: Unpublished data

SITE	USDA, Tucson Arizona, USA
IN CHARGE OF EXPERIMENTS	Fabiana Ahumada
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard liquid
DOSAGE	2 strips per brood chamber
Number of colonies	16
Number of Treatments	1
GROUPS	
• GROUP 1	1 application of HopGuard II
Period of treatment	Autumn 2013
Length of treatment	14 days
CONTROL TREATMENT	Sugar shake method

Results	Mite mortality %
• GROUP 1	86
Damages or remarks	No damage was found in any colonies
Conclusions	This was an exploratory study, but it showed a high efficacy for only a 14 day treatment. The honey samples did not contain detectable residues of hop beta acids.
Final comment	The results are encouraging.

Study 4.

Reference: Nasr et. al. HopGuard II Under-Over Dosage and Application Experiment, Summer 2014 – Not Published

SITE	Crop Diversification Centre North , Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Medhat Nasr
FORMULATIONS	Hop Beta Acid (HBA)- HopGuard II
PRESENTATION	Corrugated cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	- Half dose: ½ a strip of HopGuard II for every 5 frames of bees - Recommended dose: 1 strip of HopGuard II for every 5 frames of bees - One and one-half dose:1.5 strips of HopGuard II for every 5 frames of bees - Double dose: 2 strips of HopGuard II for every 5 frames of bees
Number of colonies	6 per treatment, 6 control
Number of Treatment GROUPS	8

- GROUP 1 Half dose applied once.
- GROUP 2 Half dose applied twice, 10 days apart
- GROUP 3 Recommended dose applied once
- GROUP 4 Recommended dose applied twice, 10 days apart
- GROUP 5 One and one-half dose applied once
- GROUP 6 One and one-half dose applied twice, 10 days apart
- GROUP 7 Double dose applied once
- GROUP 8 Control (no treatment)

Period of treatment
- GROUP 1,3,5,7 – June 16 to June 26, 2014, Finishing treatment (Apivar) removed August 7, 2014
- GROUP 2,4,6,8, - June 16 to July 6, 2014, Finishing treatment removed August 18, 2014

Length of treatment
- GROUP 1,3,5,7 - 10 days
- GROUP 2,4,6,8 - 21 days

CONTROL TREATMENT: Apivar Strips (1 strip per 5 frames of Bees)

Results	Efficacy %
• GROUP 1	33
• GROUP 2	72

- GROUP 3 34
- GROUP 4 79
- GROUP 5 36
- GROUP 6 65
- GROUP 7 46
- GROUP 8 22

Damages or remarks No damage was found in any colonies

Conclusions 2 applications of HopGuard II at any dose provided better mite control than a single application. There was no significant difference between the half dose and recommended dose when applied twice at 72% and 79% efficacy.

Final comment 2 applications as opposed to one of HopGuard II are significantly more effective at controlling Varroa mite populations. However, the 2 applications did not provide a high enough efficacy to be considered an effective miticide in the summer.

Study 5.

Theophilidis: Unpublished data

SITE	Aristotle University of Thessaloniki, Greece
IN CHARGE OF EXPERIMENTS	George Theophilidis
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips, hanging over frames 3/4 and 7/8, diagonally opposite each other
Number of colonies	8 per treatment, 4 control
Number of Treatments	4
GROUPS	
• GROUP 1	2 strips removed after 28 days
• GROUP 2	2 strips, replaced after 28 days, removed 28 days later
• GROUP 3	2 strips, replaced after 14 days, removed 14 days later
• GROUP 4	Untreated control: 2 blank strips removed 28 days
Period of treatment	Autumn 2014
Length of treatment	28 days

CONTROL TREATMENT	2 x 25 gram treatments of Apiguard with 1 week interval followed by 2 strips Apistan for 3 weeks and one application of oxalic acid when colonies became broodless
Results	Mite mortality %
• GROUP 1	78.84
• GROUP 2	91.95
• GROUP 3	87.12
• GROUP 4	10.14
Damages or remarks	No damage was found in any colonies
Conclusions	HopGuard II provided a significantly higher efficacy than the control. Two applications provided the highest efficacy.
Final comment	HopGuard II could be a good product for Varroa control.

Study 6.

Ahumada: Unpublished data

SITE	Texas and Dakota, USA
IN CHARGE OF EXPERIMENTS	Fabiana Ahumada
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard liquid
DOSAGE	2 strips per brood chamber
Number of colonies	180
Number of Treatments	3
GROUPS	
• GROUP 1	1 application, spring and autumn
• GROUP 2	2 applications at 14 day interval, spring and autumn
• GROUP 3	Control- alternative acaricide treatment
Period of treatment	Spring and autumn 2015
Length of treatment	28 days for both spring and autumn

CONTROL TREATMENT	Apivar (Amitraz) treatment
Results	Mite mortality %
• GROUP 1	95
• GROUP 2	95
• GROUP 3	90
Damages or remarks	No damage was found in any colonies
Conclusions	HopGuard II had a high efficacy but, there was no difference between 1 or 2 treatments. HopGuard II had a significantly higher efficacy than the control (Apivar).
Final comment	Excellent results from spring and autumn treatment with HopGuard II

Study 7.

Bassi: Unpublished data

SITE	Villa Luisa, Gorizia, North West Italy
IN CHARGE OF EXPERIMENTS	Dr. P. Bassi
FORMULATIONS	HopGuard II plus HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips, hanging over frames 3/4 and 7/8, diagonally opposite each other
Number of colonies	8 per treatment, 8 control
Number of Treatments	3
GROUPS	
• GROUP 1	2 HopGuard II strips replaced after 14 days, removed 14 days later
• GROUP 2	2 HopGuard strips, replaced after 7 and 14 days, removed 7 days later
• GROUP 3	Untreated control: 2 blank HopGuard II strips (no active ingredients), replaced after 14 days, removed 14 days later
Period of treatment	Summer 2015
Length of treatment	28 days

CONTROL TREATMENT	Apivar (amitraz) and Apibioxal (oxalic acid)
Results	Mean efficacy %
• GROUP 1	74.14
• GROUP 2	62.56
• GROUP 3	6.26
Damages or remarks	No damage was found in any colonies
Conclusions	HopGuard II provided moderate mite control and HopGuard was eliminated from further investigation.
Final comment	The efficacy was lower compared to other studies. The dosage should be modified.

Study 8.

Reference: Nasr, et. al. HopGuard II Concentration, Application and Honey Residues Experiment, Summer 2015 – Not Published

SITE	Crop Diversification Centre North, Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Medhat Nasr
FORMULATIONS	HopGuard II
PRESENTATION	Corrugated cardboard strips (44.4 x 3.2 cm)
DOSAGE	HopGuard II - 21g Hop Beta Acid per strip - 23g Hop Beta Acid per strip - 25g Hop Beta Acid per strip
Number of colonies	6 per treatment, 6 control, 5 for HopGuard II 25g/strip applied once
Number of Treatment	10
GROUPS	
• GROUP 1	25g/strip applied once
• GROUP 2	23g/strip applied once

• GROUP 3	21g/strip applied once
• GROUP 4	Apivar applied at second treatment date
• GROUP 5	Apivar applied at first treatment date
• GROUP 6	25g/strip applied twice, 14 days apart
• GROUP 7	23g/strip applied twice, 14 days apart
• GROUP 8	21g/strip applied twice, 14 days apart
• GROUP 9	Apivar applied at first treatment date and taken out at second treatment date
• GROUP 10	Control
Period of treatment	June 16 to 30, 2015; Finishing treatment taken out August 10, 2015
Length of treatment	28 days
CONTROL TREATMENT	Apivar Strips (1 strip per 5 frames of Bees)

Results	Efficacy %
• GROUP 1	48.8
• GROUP 2	38.1
• GROUP 3	41.6
• GROUP 4	62.3
• GROUP 5	73.0

- GROUP 6 61.9
- GROUP 7 63.4
- GROUP 8 64.1
- GROUP 9 19.8
- GROUP 10 28.5

Damages or remarks	No damage was found in any colonies
Conclusions	2 applications of HopGuard II at any dose provided better mite control than a single application. There was no significant difference between the efficacy and the concentration of the HopGuard II strip.
Final comment	2 applications as opposed to one of HopGuard II were significantly more effective at controlling Varroa mite populations. However, the 2 applications were only moderately effective at controlling the Varroa mite population in the summer. The different concentrations showed no significant difference in efficacy.

Study 9.

Theophilidis & Papachristoforou: Unpublished data

SITE	Aristotle University of Thessaloniki, Greece
IN CHARGE OF EXPERIMENTS	Prof. G. Theophilidis/Dr A. Papachristoforou
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	1 or 2 strips, inserted into the middle (Group 1) or edges (Group 2) of the winter cluster
Number of colonies	19 broodless colonies
Number of Treatments	3
GROUPS	
• GROUP 1	1 HopGuard II strip, removed after 14 days
• GROUP 2	2 HopGuard II strips, removed after 14 days
• GROUP 3	Untreated control: 1 blank strip, removed after 14 days
Period of treatment	Winter 2015
Length of treatment	28 days

CONTROL TREATMENT	Two applications of oxalic acid with 14 day interval
Results	Mite mortality %
• GROUP 1	87.41
• GROUP 2	94.99
• GROUP 3	9.37
Damages or remarks	No damage was found in any colonies.
Conclusions	Both HopGuard II treatments had high efficacy however, there was no differences between the treatments.
Final comment	1 strip works as well as 2 strips per hive.

Study 10.

Bassi: Unpublished data

SITE	Villa Luisa, Gorizia, North East Italy
IN CHARGE OF EXPERIMENTS	Dr. P. Bassi
FORMULATIONS	HopGuard II plus HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips, inserted through colony cluster diagonally opposite each other.
Number of colonies	8 per treatment, 8 control
Number of Treatments	3
GROUPS	
• GROUP 1	2 HopGuard II strips replaced after 14 days with 1 strip, removed 14 days later
• GROUP 2	2 HopGuard strips, replaced after 7 and 14 days, removed 7 days later
• GROUP 3	Control- 2 blank HopGuard II strops (no active ingredient), replaced after 14 days, removed 14 days later
Period of treatment	Winter 2015/2016
Length of treatment	28 days

CONTROL TREATMENT	Apivar (amitraz) and Apibioxal (oxalic acid)

Results	Mean efficacy %
• GROUP 1	95.02
• GROUP 2	96.03
• GROUP 3	9.04

Damages or remarks	Treatment resulted in some adult bee loss in small, wintering colonies.
Conclusions	HopGuard II provided excellent Varroa control in the winter.
Final comment	HopGuard II has a high efficacy but the dosages should be changed.

Study 11.

Belletti: Unpublished data

SITE	Boscat, Gorizia, North East Italy
IN CHARGE OF EXPERIMENTS	Dr. PA Belletti
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	One strip, inserted through colony cluster and removed after 14 days.
Number of colonies	8 per treatment, 8 control
Number of Treatments	1
GROUPS	
• GROUP 1	1 HopGuard II strip during 14 days.
Period of treatment	Winter 2015/2016
Length of treatment	14 days
CONTROL TREATMENT	Apivar (amitraz) and Apibioxal (oxalic acid)

Results	Mean efficacy %
• GROUP 1	96.01

Damages or remarks	No damage was found in any colonies.
Conclusions	HopGuard II provided excellent Varroa control in the winter.
Final comment	HopGuard II has a high efficacy when adapted for winter conditions.

Study 12.

Reference: Nasr, et. al. HopGuard II Concentration, Application and Honey Residues Experiment, Spring 2016 – Not Published

SITE	Crop Diversification Centre North , Edmonton, Alberta, Canada
IN CHARGE OF EXPERIMENTS	Medhat Nasr
FORMULATIONS	Hop Beta Acid (HBA)- HopGuard II
PRESENTATION	Corrugated cardboard strips (44.4 x 3.2 cm) containing 3.84 g of a 16% Hop Beta Acid
DOSAGE	- Recommended dose: 1 strip per 5 frames of bees - Double dose: 2 strips per 5 frames of bees - Single application: only applied once - Double application: applied twice, 10 days apart
Number of colonies	5 per treatment, 5 control
Number of Treatments	5
GROUPS	
• GROUP 1	HopGuard Recommended dose Double application
• GROUP 2	HopGuard Double dose Double application

• GROUP 3	HopGuard Recommended dose Single application
• GROUP 4	HopGuard Double dose Single application
• GROUP 5	Control (no treatment)
Period of treatment	May 6-July 14, 2016
Length of treatment	Single application- 10 days, Double application- 21 days
CONTROL TREATMENT	Apivar Strips (1 strip per 5 frames of Bees) for 42 days

Results	Efficacy %
• GROUP 1	62
• GROUP 2	65
• GROUP 3	29
• GROUP 4	37
• GROUP 5	~20

Damages or remarks	No damage was found in any colonies
Conclusions	An early spring resulted in brood begin present during the experiment. Single applications of HopGuard II were ineffective at controlling Varroa. A double application was more effective, though there was no difference between using the recommended dose or the double dose.

Final comment	HopGuard II is not an effective mite control in the presence of brood. Two applications are necessary to control mites.

Study 13.

Vidondo: Unpublished data

SITE	Glaban Apiary, Tandil, Buenos Aires, Argentina
IN CHARGE OF EXPERIMENTS	Patricio Vidondo
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips hanging over frames 3 and 7
Number of colonies	16
Number of Treatments	3
GROUPS	
• GROUP 1	2 HopGuard II strips, replaced on day 15
• GROUP 2	2 HopGuard II strips, replaced on day 21
• GROUP 3	2 HopGuard II strips, replaced on day 28
Period of treatment	28th March – 6th June 2016
Length of treatment	56 days

CONTROL TREATMENT	Amivar 500 (Amitraz), 14 day treatment

Results	Mite mortality %
• GROUP 1	92.4
• GROUP 2	92.2
• GROUP 3	98.0

Damages or remarks	No damage was found in any colonies
Conclusions	The application of a second dose is needed and should be done on day 28.
Final comment	The results support the use of HopGuard for Varroa control.

3. Non-Safety Adverse Effects *(DACO 10.3; M10.3)*

This portion of the value package presents all information related to non-safety adverse effects (e.g., phytotoxicity to the host or rotational crop, damage to the site of application, etc.). This may include experimental results from research trials, published scientific literature, scientific rationales, and use history information.

3.1. General Factors Influencing Non-Safety Adverse Effects

Describe any general factors that may influence non-safety adverse effects, such as:

- environmental and edaphic conditions
- application timing relative to crop or pest growth stage
- description of other pest management strategies used during the study that could be relevant to potential adverse effects

N/A

Summary Studies done during early stages USA Hop Beta Acid (HopGuard) on bee toxicity. See Section 2.4 for additional studies.

Study 1.

Theophilidis & Papachristoforou: Unpublished data

SITE	Aristotle University of Thessaloniki, Greece
IN CHARGE OF EXPERIMENTS	George Theophilidis and Dr. Alex Papachristoforou
FORMULATIONS	HopGuard II
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips, hanging over brood frames 3/4 and 7/8, diagonally opposite each other and double dose, 4 strips positioned similarly.
Number of colonies	6 replicates per treatment group, 6 controls (18 total)
Number of Treatments	3
GROUPS	
• GROUP 1	2 HopGuard II strips, replaced after 14 days then removed 14 days later
• GROUP 2	4 HopGuard II strips, replaced after 14 days then removed 14 days later

• GROUP 3	Control: 2 blank HopGuard II strips (no active ingredient), replaced after 14 days then 14 days later
Period of treatment	Summer 2015
Length of treatment	28 days
CONTROL TREATMENT	Not required
Results	Overdosing (Group 2) resulted in higher initial bee losses for the first 48 hours at which time the effect subsided. No difference in bee mortality or bee behaviour was observed between Group 1 and Control.
Damages or remarks	With 4 strips per hive, the bees were subjected to excessive wetting which can cause bee mortality. 2 queens were lost in overdosed colonies. No other behavioural aberrations were observed.
Conclusions	Bee mortality with the normal hive treatment (2 strips per hive) was not significantly different to Control. HopGuard II was generally well-tolerated, even at double the recommended dose but within the first 48 hours post application, more adult bees died using the higher dose than Control. After 48 hours, no further disturbance was evident in the overdosed hives or in any other hive.
Final comment	Two HopGuard strips per hive application are recommended.

3.2. Supporting information from earlier formulations of the product or similar Products

If information is available for product formulations tested during earlier stages of development, or similar products, rationales and bridging data should be presented in this section to demonstrate equivalence between the products.

Experimental data from trials that evaluated both formulations in the same trial should be presented in this section using a format similar to that presented above in Section 2.4 Support for Proposed Claims.

To avoid duplication, a reference to Section 2.2 may be presented here, and all information concerning previous or similar formulations as it relates to both efficacy and non-safety adverse effects can be presented in Section 2.2.

Summary Studies done during early stages USA Hop Beta Acid (HopGuard) on bee toxicity. See Section 2.2 for additional studies.

Study 1.

Rademacher: Unpublished data

SITE	Free University of Berlin, Germany
IN CHARGE OF EXPERIMENTS	Dr. Eva Rademacher
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	2 strips hanging over the frames into the winter cluster
Number of colonies	18, derived from nuclei colonies
Number of Treatments	2
GROUPS	
• GROUP 1	2 strips each
• GROUP 2	Untreated control (8 colonies)
Period of treatment	Winter 2010-11

Length of treatment	17 days
CONTROL TREATMENT	Oxuvar
Results	Bee mortality %
• GROUP 1	7.2
• GROUP 2	0.7
Damages or remarks	Mite mortality was significantly higher in treated colonies than in the untreated control.
Conclusions	The 17 day treatment with HopGuard® strips was generally well tolerated by the bees. However, bee mortality was significantly higher
Final comment	Hopguard II could be a potential product for Varroa control.

Study 2.

Rademacher: Unpublished data

SITE	Free University of Berlin, Germany
IN CHARGE OF EXPERIMENTS	Dr. Eva Rademacher
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	One strip per colony with a mean dosage of 260 µg per bee
Number of colonies	3
Number of Treatments	2
GROUPS	
• GROUP 1	HopGuard® strip placed on neighbouring comb to brood comb
• GROUP 2	Untreated control
Period of treatment	June 6-24, 2011
Length of treatment	18 days
CONTROL TREATMENT	Oxuvar

Results	Brood mortality %
• GROUP 1a	39.6
• GROUP 1b	6.5
• GROUP 2	17.7
Damages or remarks	The first five days of a treatment with HopGuard can cause a significant reduction in the number of brood in cells near the treatment strip. The brood combs away from the treatment strip showed brood mortality compared to the rest of the colony.
Conclusions	For a treatment during brood rearing, the HopGuard strip should be placed on brood free comb with one comb in between treatment and brood comb.
Final comment	HopGuard strips should be placed on brood free comb.

Study 3.

Rademacher: Unpublished data

SITE	Free University of Berlin, Germany
IN CHARGE OF EXPERIMENTS	Dr. Eva Rademacher
FORMULATIONS	HopGuard
PRESENTATION	Cardboard strips soaked in HopGuard® (16% w/w beta acids)
DOSAGE	Portion of HopGuard® strip pinned on to a piece of comb
Number of colonies	2 x 3 small cages with 50 bees each per concentration, 1500 bees total
Number of Treatments	5
GROUPS	
• GROUP 1	9.8 µg beta acids per bee (3.52 cm² strip per cage)
• GROUP 2	12.5 µg beta acids per bee (4.48 cm² strip per cage)
• GROUP 3	15.2 µg beta acids per bee (5.44 cm² strip per cage)
• GROUP 4	20.5 µg beta acids per bee (7.36 cm² strip per cage)
• GROUP 5	Untreated control
Period of treatment	September – November 2011

Length of treatment	Bee mortality was monitored for 72 hours after treatment
CONTROL TREATMENT	Portion of plain cardboard strip pinned on to a piece of comb
Results	Bee mortality %
• GROUP 1	1.0
• GROUP 2	2.0
• GROUP 3	1.0
• GROUP 5	0.4
Damages or remarks	The dermal application of individual trickling of HopGuard® on to the bees was not tolerated by the bees, probably caused by the moistening agent. The mortality was significantly increased compared to the control group. Therefore it was necessary to return to the posology of the final product for the dose-response-relationship test in the laboratory.
Conclusions	The treatment with HopGuard® strips in different dosage was well tolerated by the bees. The mortality was in the range of the corresponding control group, no significant differences occurred.
Final comment	It was not possible to establish a dose-response relationship for bee mortality using HopGuard® strips.

Study 4.

Ahumada-Segura: Unpublished data

SITE	USDA-ARS Carl Hayden Bee Research Centre Apiary, Tucson, AZ
IN CHARGE OF EXPERIMENTS	Fabiana Ahumada-Segura
FORMULATIONS	Hop Beta Acid (HBA)
PRESENTATION	Cardboard strips (44.4 x 3.2 cm)
DOSAGE	2 strips per hive
Number of colonies	10 treatment, 10 control (20 total)
Number of Treatments	2
GROUPS	
• GROUP 1	Untreated control
• GROUP 2	3.84 g of a 16% HBA solution on cardboard strips
Period of treatment	
Length of treatment	3 days
CONTROL	
TREATMENT	

Results	Average number of dead bees before/after treatment
• GROUP 1	N/A- not significantly different
• GROUP 2	6.9/8.2
Damages or remarks	No damage was found in any colonies
Conclusions	No significant increase in bee mortality during sampling period.
Final comment	

3.3. Support for Proposed Claims *(DACO 10.3.2; M10.3.1)*

3.3.1. Host Crop Claims

Each claim should be identified (i.e., each application timing, method of application, pest, tank mix, etc.), and the approach and information used to support each of these claims, with respect to non-safety adverse effects, should be clearly indicated.

Claims may be organized in subsections by host crop, application timing/method, etc., whichever is best suited to the specific claims being proposed. A description of the effects of the treatment on the crop or use site and whether the effects were permanent or temporary, should be included.

Each subsection should present all the various types of information available (i.e., experimental results from research trials, published scientific literature, scientific rationales, and use history information) to support the claims within the subsection. It is important to note that this section of the value summary consists of a synopsis of all supporting information on non-safety adverse effects and not a compilation of the individual reports. Copies of individual reports should be submitted as a separate component of the value information package.

When crop tolerance or use site research trials are presented, a summary of the host/site reactions to the treatment as they relate to the use pattern employed in the studies should be included. Any differences

between the tested use pattern and the proposed use pattern should be explained. Individual trial reports should be included in the value package.

When published scientific literature is presented, an explanation of the relevance of the results to the proposed use should be provided. A comparison of the proposed use pattern and the use pattern employed in the studies should be included. All units should be expressed in metric to enable a direct comparison of the application rates. If the products tested in the study have equivalent products registered in Canada, they should be identified. Any unexpected results should be discussed. A copy of the published articles should be included in the value package.

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When use history information is presented, a summary of the information in the templates as it relates to non-safety adverse effects on the host or use site should be provided. This should include (1) a brief explanation of the similarities of the proposed use pattern and the registered use pattern in the foreign jurisdiction, (2) information on the crop/use site tolerance profile of the proposed use, and (3) validation information describing the source of the information and the extent of their direct experience with the product.

When scientific rationales are presented, a clear explanation of the basis for support of the proposed use should be provided. It should contain the appropriate level of detail to enable a conclusion regarding the proposed claims to be made.

Regardless of the type of information submitted, the applicant should provide an explanation if the use pattern in the supporting information submitted differs from the proposed use pattern.

The subsection should finish with the presentation of an overall conclusion made on the basis of an integration of all the types of information presented to support the claim.

3.3.2. Rotational Crop Claims *(DACO 10.3.3; M10.3.1)*

Each claim should be identified (i.e., each rotational crop and its proposed replanting interval relative to application), and the approach and information used to support each of these claims, with respect to non-safety adverse effects, should be clearly indicated.

N/A

4. Consideration of Benefits *(DACO 10.5; M10.4)*

Canada has approximately 9,800 beekeepers and 750,000 honey bee colonies in 2016. The value of Canada's honey industry has been estimated to be $231 and $158 million in 2015 and 2016, respectively (Statistics Canada, 2015). The value of honey bees to Canada's agricultural economy, including pollination of crops, high value cash crops and minor crops, is estimated to be over $2 billion.

The economic losses associated with Varroa mites are serious. In Canada the long term average colony mortality is reported to be approximately 15% (Pernal 2007). The colony mortality in Canada ranged from 30%-35% from 2007 to 2010. The range of colony mortality has decreased by 40-50% since 2011. The

reduction in mortality is likely related to better control of Varroa mites using Apivar and the improvement of beekeeping management practices.

Based on the above information, Canadian beekeepers lost 600,000 honey bee colonies between 2007 and 2009, which is equivalent to 100% of the colonies in Canada mainly due to the failure of controlling Varroa mites. As a result, beekeepers have incurred additional costs for purchasing or making up replacement colonies and loss of crops. A study conducted by Alberta Agriculture and Rural Development in 2007 revealed that the economic loss experienced by the Alberta beekeeping industry was estimated to be between $16.7 and $24.6 million as a result of increased colony losses from 15% to 30% (Chaudhary and Nasr, 2007). A recent study in Alberta also showed that the potential economic losses could be as high as $70 million/year due to high prices of honey, bees and pollination services' fees. In Canada, the estimated economic impact of losing 30% of honey bee colonies in one year was calculated to be valued at approximately $200 million. The impact of lack of bees for crop pollination and yield would be very significant.

If Apivar fails, it will likely lead to high losses of bee colonies and a decreasing number of healthy honey bee colonies available for crop pollination. Indeed, more demand than supply was evident for pollination in British Columbia during the spring of 2008. Some blueberry pollination contracts could not be honoured and some Alberta beekeepers, who winter their bees in British Columbia, delayed moving their bees to help supplying bees to fill in these pollination contracts. In Alberta, beekeepers who have never moved bees for hybrid canola pollination rented their bees in 2008 to help meet the demand for canola pollination. Table 7 is a summary of the estimated economic impact on honey yield and pollination value when Varroa mites are not adequately controlled.

Consideration of the Pest Management Regulatory Agency (PMRA) to register HopGuard® II is very important to the Canadian beekeepers. Beekeepers will have access to a new and effective bee protection tool. Varroa mites have not been exposed to this miticide. Consequently, Varroa mites don't have known resistance to this product. This product will fit in the Varroa integrated management system and provide another option to beekeepers. Thus, HopGuard® II will help to maintain healthy bees and a sustainable, competitive industry that can supply an adequate number of bee colonies for crop pollination.

4.1. Survey of Alternatives *(DACO 10.5.1; M10.4.3)*

A list of conventional and non-conventional (e.g. biological pesticides) products registered in Canada for the same uses currently being proposed. If the current alternatives do not address grower/user needs, an explanation should be provided. The date when the search was conducted should be reported.

- Varroa mites were first discovered in Canada in 1989. Honey bee colonies with this mite were destroyed in 1989 and 1990 because there were no registered chemical controls.
- **Apistan:**
    - In 1991, the Emergency Use Registration (EUR) of the synthetic pyrethroid acaricide, Apistan® (fluvalinate) to control the *Varroa* mite was granted. Apistan® was given full registration in 1993 (Clay 1996). It is believed that the enormous negative impact

of Varroa mites on honey bees was averted in Canada because of early registration and adoption of an effective mite control product like Apistan®.

- Until early 2000, the Varroa mite had been successfully controlled in Canada primarily using Apistan®. To a lesser extent some beekeepers were using formic acid, but with mixed results. In 2001, a population of Varroa mites resistant to the active ingredient fluvalinate (Apistan®) was discovered in Canada. Over the next four years, Varroa population testing for fluvalinate resistance (Pettis et al. 1998), showed that fluvalinate-resistant Varroa mites had become widespread across Canada. The development of strains of Varroa mites resistant to fluvalinate had also been documented in a number of countries including the United States.

- **CheckMite+:**

  - In response to the impending threat that treatment-resistant Varroa mites posed on the Canadian beekeeping industry, in 2002 the Pest Management Regulatory Agency (PMRA) granted an Emergency Use Registration (EUR) of CheckMite+™ Beehive Pest Control Strip (10% coumaphos) for affected provinces. Similar to the fluvalinate-resistant Varroa mites, testing for coumaphos resistance showed that Varroa mites had developed resistance to coumaphos as early as 2007 in some Canadian provinces.

  - In 2008 Varroa mites resistant CheckMite+™ were reported in Canada.

  - In fact Varroa resistant to CheckMite+ and Apistan® were considered widespread. These two miticides currently don't address beekeepers' needs.

- **Apivar:**

- In August 2008, at the request of the Canadian Honey Council and provincial governments, PMRA approved the EUR of Apivar® for the control of Varroa mites in honey bee colonies. In 2012, Apivar was granted conditional registration for use in Canada until December 31, 2018.
- Since then, beekeepers using Apivar® in spring or fall, have found it to have very good efficacy. Often the mite control is good enough that the colonies typically will need a single treatment during the bee season. In some cases, alternative mite control options have been used as a supplementary treatment when needed. This practice is used to slow down the development of resistance as part of a resistance development management strategy.

- **Thymovar:**
  - Is a registered product. It is an individual dose of Thymol in wafer. It requires ambient temperature (15-35°C)
  - The efficacy ranges between 74-95% when applied and temperature's requirements are met.
  - In Canada due to cold spring and variable temperatures during fall, the efficacy of the product is not predictable.
  - May reduce queen egg-laying activity; may increase adult and young larvae mortality; works best under warmer temperatures; may cause bees to beard in hot weather; human skin irritant.
  - It can be used as an option in an Integrated Pest Management Program. However, monitoring and repeat application might be needed.

- **Mite-Away Quick Strips® (MAQS®)**

    - MAQS®: saccharide gel strip in a laminated paper wrap formulation of 46.7% formic acid. It is recommended to use when outside day temperature 10-33ºC.

    - In northern climates, the window of opportunity for effective formic acid treatment in the spring and fall has been extremely limited. MAQS™ has resolved a lot of the temperature dependency associated with using formic acid; however because of the colony size requirements for using MAQS™, it is estimated that this product may not work in up to 30% of colonies in some provinces at critical treatment periods in spring and fall.

- **65% formic acid liquid**

    - In Canada 65% Formic acid liquid is permitted to be applied in soaked absorbing pads, slow release pads or Mitegone pads

    - Treatment options are Absorbing pad (Dri-Loc) (30-40 ml per 2 story hive) up to3 applications: one every 7-10 days; Slow release pad (250ml) once, Mitegone (120-125 g formic acid 65% per pad), one pad per 5 frames of bees; 2 times per year.

    - It is recommended to use when outside temperatures are between 10°C and 30°C, and hive entrances are fully open.

    - It has similar limitation as MAQS®

- **Oxalic acid**

- Sugar syrup drip with a syringe or drenching applicator, also Sublimation.
- It can be applied when populations increase in spring and populations decrease in fall when there is little brood and brood rearing is reduced.
- Efficacy range is 82-99%, especially if there is no brood present in hives.
- Requires 2-3 applications.

4.2. Compatibility with Current Management Practices Including IPM *(DACO 10.5.2; M10.3.2, M10.3.2.1, M10.3.2.2, M10.4.4)*

A description of how the proposed use can be integrated into the production system, including its contribution to integrated pest management. For example, information regarding pesticide spray programs that incorporate the proposed use or operational trials in actual grower fields could be provided, when available. In addition, any recommendations by extension personnel or information demonstrating the product's role and contribution to sustainability, such as the use of the product in IPM programmes, should be included. Information on any potential adverse effects on beneficial insects, as it affects sustainability of the use, should be explained.

Varroa mites have developed resistance to most registered synthetic miticides i.e. Apistan and CheckMite+. The only synthetic miticide known to be effective is Apivar. This miticide has been used since 2008 by beekeepers in spring and fall to control mites. Although the efficacy of Apivar has been acceptable in Canada, beekeepers are worried about merging resistance at any time.

Canadian beekeepers also have access to registered organic acids and thymol based miticides (i.e. formic acid, oxalic acid and Thymovar). Formic acid has been available for beekeepers to use to control Varroa mites since the mid 1990's (Note to CAPCO C94-05) and subsequently Mite-AwayII™, MAQS, oxalic acid and Thymovar since the mid-2000's. Although formic acid, oxalic acid and thymol based treatments have been available for a number of years these products have never been well adopted by beekeepers. In addition to the user safety concerns regarding the use of strong acids in their hives, many beekeepers have been concerned with the negative impact the product could have on the bees if too much or too little of

these products was released in the hive. Beekeepers have also been concerned with the variable levels of efficacy based on time of treatments, ambient temperature during treatment time and the presence of brood.

Imdorf, et. al. (1990) reported that optimum efficacy of formic acid based products is obtained when the ambient temperature is 18-25°C, with minimum night temperature no lower than 12°C during the application period. Ostermann and Currie (2004) reported greater consistency in formic acid evaporation is achieved when the ambient temperature outside the hive is greater than 15°C. Temperatures during formic acid application in bee colonies are critical for achieving adequate control of Varroa (Calderone and Nasr, 1999). Mite mortality was only 56% when the daily average released formic acid was below the recommended level (≥10 ml/day) during a fall treatment in upstate New York.

Given that oxalic acid should not be used when brood is present in the hive, it would be an understatement to say that this is a severe limitation for this product and that it cannot be relied upon to control a Varroa mite outbreak prior to the broodless period (i.e. late fall, winter or early spring). When oxalic acid is applied to colonies that contain brood, the efficacy is reduced by 25% (Marcangeli and Garcia, 2004). Therefore, beekeepers need to treat colonies during the broodless period in late fall/winter to achieve high efficacy using oxalic acid.

Thymovar is a thymol based product. It volatilizes and accumulates in bee hives when the ambient temperature is between 15-20°C. Once the thymol vapour is at a high enough concentration, it is more toxic to mites, but is not harmful bees. When the ambient temperature is over 20°C, the thymol vapour drives the bees away from the brood cluster. Therefore, the queen would stop egg- laying and the brood production can be reduced by up to 25%. This reduction in brood production will consequently reduce the number of bees in bee colonies and decrease the honey production.

4.3. Resistance Management *(DACO 10.5.3; M10.4.4)*

A description of how the proposed use contributes to resistance management, in consideration of other registered alternatives. Information on the following should be provided: resistance risk of the pesticide active ingredient, reports of resistance in the target pest, effectiveness of the product on pests, whether the product represents a new pesticide mode of action for the crop, and whether the product can be incorporated in a resistance management strategy as a tank mix partner or a rotational product. In addition, any reports on baseline sensitivity and resistance monitoring should be provided, if they are available.

Based on the mode of action of Potassium Salts of Hop Beta acids as a contact miticide that may cause damage to mites leading to killing mites, the development of resistance to HopGuard II is unlikely in Varroa mite populations.

4.4. Contribution to Risk Reduction *(DACO 10.5.4; M10.4.4)*

A description of how the proposed use contributes to risk reduction, in consideration of other registered alternatives. Discuss how the use of the product contributes to risk reduction. For example, use of the product may reduce reliance on chemical alternatives. If the proposed use is considered a replacement for a use that is being phased out through re-evaluation, it should also be stated in this section.

Canada has approximately 9000 beekeepers and 725,000 honey bee colonies. The value of Canada's honey industry is estimated to be worth approximately $157-$210 million (Statistics Canada, 2016). The value of honey bees to Canada's agricultural economy, including crop pollination, is estimated to be over $2 billion. The long term average colony mortality in Canada has been approximately 15%. When the Varroa treatment failed in 2007-2010, honey bee colony mortality averaged 30% (CAPA National Survey, 2016). Investigating the possible causes of the failure to control mites showed that Varroa had developed resistance to CheckMite+. Thus, the PMRA actively responded to beekeepers' demands and registered Apivar to control Apistan and CheckMite+ resistant strains of Varroa mites in 2008. Since then, beekeepers have relied on Apivar for Varroa treatment. Consequently, the bee colony mortality decreased by 50% since 2011.

Based on the above information, Canadian beekeepers have lost 600,000 honey bee colonies from 2007 to 2011, which is equivalent to 83% of the colonies currently in Canada. Once beekeepers were able to successfully controlled Varroa mites, bee mortality was reduced to about 20%. This reduction in winter loss can likely be attributed to better control of resistant Varroa mites by using Apivar.

In addition to the potential loss of production from these dead colonies, beekeepers have also incurred additional costs for purchasing or making up replacement colonies. Beekeepers who divide their colonies to make new colonies to replace dead ones end up weakening their honey producing colonies. Consequently, honey production can be greatly affected. A study conducted by Alberta Agriculture and Rural Development in 2007 revealed that the economic loss experienced by the Alberta beekeeping industry was estimated to be between $16.733 and $24.655 million. In Canada, the estimated economic impact of losing 30% of the honey bee colonies in one year was calculated to be valued at $30-45 million (Chaudhary and Nasr, 2007).

Successive annual colony losses by Canadian beekeepers at levels exceeding the long term average of 15% are unsustainable. This situation would likely decrease the number of healthy honey bee colonies available for crop pollination. Table 2 is a summary of the estimated economic impact on honey yield and pollination value when Varroa mites are not adequately controlled.

Table 2. Potential Economic Impact of not adequately controlling Varroa mite.

Region	**Canada**
Number of bee hives	725,000
Number of beekeepers	7000
Value of cash Farm receipts	$157 Million
Value of contribution to pollinated crops	$2 Billion
Average honey production/hive	133 lbs(61kg)
Expected yield without treatment	93 lbs (42kg)
Expected yield with treatment	100 %
Average winter mortality with effective treatment	18%
Average winter mortality without effective treatment or use of proposed HopGuard II registered product	30% = 217,500

Expected average winter mortality with treatment including using HopGuard II registration	18%
Estimated crops loss without using proposed HopGuard II registered product	30%
Estimate of Honey and Hive Products Value without using proposed HopGuard II registered product	$110 Million
Estimate of Honey and Hive Products Value losses without using proposed HopGuard II registered product	$47 Million
Estimate of Pollinated Crop Value without using proposed HopGuard II registered product	$1.4 Billion
Estimate of Pollinated Crop Value losses without using proposed HopGuard II registered product	$600 Million

HopGuard® II is a valuable tool for controlling Varroa mites in honey bee colonies. Data demonstrated that HopGuard® II can provide 75- 98% control of Varroa mites when two consecutive applications are applied, 10 days apart in spring or fall. High Varroa control can be achieved when HopGuard® II is used, when little or no brood is present in the hives (i.e. in the late fall to early spring). Moreover, HopGuard® II will eventually contribute to sustainability for Varroa control, as it is compatible with current control products. It will be an important tool for enhancing the sustainability of Varroa mite management in bee colonies, as resistance is unlikely to occur with this product. It will also improve the management program for pesticide resistance by adding an alternative mode of action to those currently used.

4.5. Social and Economic Impacts *(DACO 10.4; M10.4.2)*

This section should describe any social or economic impacts associated with the proposed use such as effects on the sustainability of the sector or trade implications (i.e. Maximum Residue Limit, or MRL, issues; impact on competitiveness of Canadian growers). Information explaining why the product is needed as well as how and to what extent product registration would benefit Canadian users should be provided. Elements such as crop value (farm-gate, market value), acreage devoted to crop, influence of the pest on crop quality and marketability, additional costs associated with the pest presence (e.g. drying costs for grain), indirect effects of the pest on the crop (e.g. alternate host for a crop disease), or priority status of proposed use according to the Grower Priority database, etc. could be considered. It would be useful to indicate if the use is an identified grower priority or whether the registration of the product would result in harmonization with US.

Information on indirect benefits that could result from the proposed use could also be included. An example is a use that could reduce overall fuel costs or reduce soil compaction. Additional information such as an attribute that contribute to a product's value could be included. For example, if a product is stable for longer periods without the need for refrigeration, this attribute could impact product cost.

Honey bees play an important role in the environment and agriculture. Every third bite of food is the product of honey bee pollination. In recent years when the honey bee colony mortality doubled due to what is known as Colony Collapse Disorder and failure of Varroa controls, the public showed concerns due to lack of honey bees for crop pollination which in turn could threaten our food supply.

In order to address bee health, beekeepers need to apply miticides at the recommended dosage to control Varroa in bee colonies. The application of these miticides is proven to be generally profitable in beekeeping. Their use has improved bee health and reduced bee colony mortalities. For example when mites developed resistance to Apistan and Checkmite+, the bee colony mortality doubled (30% per year) between 2007 and 2011. Once beekeepers used Apivar to treat resistant strains of Varroa mites, the annual mortality was reduced to 15-18%, which is acceptable long-term mortality.

Overall, beekeepers have been able to reduce the cost of production by reducing the cost of replacing dead colonies and increasing their honey production. This is mainly because they have been able to effectively control Varroa mites. This reinforces the view that using effective alternative means for Varroa control in an integrated system is important in order to have an environmentally sound system for beekeeping.

4.6. Health, Safety and Environmental Benefits *(DACO 10.5.5; M10.4.4)*

This section is a summary of any potential health, safety or environmental benefits that could result from the proposed use of the pesticide. It is not a summary of the information provided to support the human health or environmental risk assessment. Where applicable, information on the benefits of the proposed use should be provided. For example, the applicant could indicate that the proposed use seeks to control a poisonous plant, a plant disease with harmful effects on humans/livestock (e.g., ergot) or control an invasive species. A product with a higher potential for crop safety (lower phytotoxicity) or a broader spectrum of activity on pests would be beneficial. There could also be references indicating if the product could replace, or reduce applications of, chemistries with health or environmental concerns.

Because there is a possibility of accidental dermal exposure by workers, individuals should observe all precautionary and first aid statements on the product label. The label-required wearing of personal protective equipment including goggles, face shield, or safety glasses by workers. Based on the limited use pattern to bee hives, bystanders are not expected to be exposed to Hops Beat acid salts.

No environmental risk-reduction measures are required. Standard label statements to protect the environment are required.

Environment benefits

- HopGuard® II seeks to preserve the health of honey bee colonies by controlling a destructive parasite, thereby preserving the ecological services that honey bees provide, namely: pollination which is fundamental to production of many food crops and reproduction of non-food native plants in our natural ecosystems.

- HopGuard® II will be an invaluable addition as a rotational product to mitigate the development of resistance in mite populations to currently registered chemical control options.

- Used and unused strips are non-hazardous to the environment and can be disposed or in landfill. Some other miticides cannot be disposed of this easily.

- Hopguard® II may serve to replace older chemistries such as Coumaphos which residues in hives have been shown to be detrimental to honey bee health.

- A thorough coverage of the absence of environmental effects by HopGuard® II, including extensive scientific references, is provided in our Section 3 EPA application (reference 1), Non-target Effects Waivers for the Technical Grade Active Ingredient Potassium salts of hop beta acids and the End Use Product HopGuard® This document provides 7 waiver requests for the Technical Grade Active Ingredient, Potassium Salts of Hop Beta Acids, and for the End Use Product-HopGuard®.

Human Health and Safety

- Beta acids are the active ingredient in HopGuard® II. They are non-toxic to humans. They are considered low risk-miticides (bio-pesticide) that can be safely used on crops.

- HopGuard® II presents a reduced risk to handler safety compared to popular organic acid-based treatments in terms of inhalation risk, and HopGuard® II is not expected to affect nerve function.
- The active ingredient is naturally found in some foods. Residues in honey are less concerning that synthetic miticide alternatives.

- A thorough coverage of the absence of health and human safety effects by HopGuard® II, including extensive scientific references, is provided in our Section 3 EPA application (reference 2), Health Effects Waiver Rationale for the Technical Grade Active Ingredient, Potassium Salts of Hop Beta Acids and the End Use Product-HopGuard®. This document provides 13 waiver requests for the Technical Grade Active Ingredient, Potassium Salts of Hop Beta Acids, and 13 waiver requests for the End Use Product-HopGuard®.

Summary and Conclusions

Honey bees play an important role in agroecosystems because they pollinate one-third of agricultural crops. In Canada, beekeepers keep over 725,000 bee colonies. Their contributions as managed pollinators are indispensable. It is estimated that the value of bee pollination to the Canadian economy is over $2 billion per year. These honey bees currently experience a suite of health threats, including colony collapse disorder and the ectoparasitic mite, *Varroa destructor* (Anderson & Trueman, 2000). Beekeepers have been struggling to control Varroa mites. Monitoring and Integrated pest Management are necessary tactics to control Varroa. This includes the use of genetically tolerant Western honey bees and control methods such as essential oils, organic acids, and synthetic miticides.

In Canada, three synthetic miticides are currently registered for Varroa control. These miticides include Apistan, CheckMite, and Apivar. Despite the risks associated with using synthetic miticides, such as residues and developing resistance, they remain the only constant method of managing Varroa mites. Essential oil and organic acid based miticides are also used as control agents for Varroa. Although they are alternative tools for Varroa control, their efficacy is variable and depends on the ambient temperature.

In order to improve the Integrated Pest Management Strategy and manage resistance to used miticides for Varroa mite control, it is recommended to use miticides with different modes of action when necessary. It was recently discovered that a natural compound from the hop plant, *Humulus lupulus*, is shown to be effective against Varroa mites. Research in the USA, Canada, and other countries has shown hop beta acid salt can be used as a new mite control agent. Following several years of laboratory and colony testing, a formulation was refined and the final product, HopGuard II, based on hop beta acids, was registered as a Varroa treatment in the USA in 2015. The efficacy ranged from 80-95% based on the presence of brood in bee colonies. It is a contact miticide, but the mode of action is unknown. In evaluating the impacts of HopGuard II on bees, there were no significant effects on brood production and queens. Based on studies in Saskatchewan and Alberta, Canada, two applications of HopGuard II (1 strip/5 frames covered with bees, 10 days apart) yielded a high efficacy of 95-98%, with no adverse effects on the honey bee colonies. Similar results were reported in South Texas, USA.

Overall, HopGuard II represents another option to add to the Varroa control tool box. Beekeepers can integrate this product into their spring and fall Varroa treatment of bee colonies. This product has a different mode of action and can be used for the management of Varroa mites that are resistant to synthetic products. Thus, beekeepers will be able to efficiently control Varroa mites and successfully reduce bee mortality.

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