SUBLETHAL EFFECTS OF THREE ACARICIDE TREATMENTS ON HONEY BEE (*APIS MELLIFERA* L.) COLONY DEVELOPMENT AND HONEY PRODUCTION

by

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ABSTRACT

Honey bee (*Apis mellifera* L.) colonies infested with the parasitic mites *Acarapis woodi* or *Varroa jacobsoni* require acaricidal treatment to control mite infestations to maintain the health and productivity of the colony. This study investigated the effects of the three acaricides fluvalinate (formulated as Apistan®), menthol, and formic acid on honey bee colony development and honey production. All acaricidal treatments were applied according to recommended and legal methods. Effects on honey bees of in-hive acaricide treatments were measured by examining a number of colony variables. In the 1995 experiment, worker bee longevity, colony weight gain, adult bee mortality, brood viability, sealed brood area, returning foragers, pollen load weight and emerged bee weight were not statistically different between fluvalinate- and formic acid-treated colonies and control colonies. However, formic acid-treated colonies experienced the lowest longevity among the three groups in the experiment. In the 1996 experiment, formic acid-treated colonies produced, on average, the lowest amount of sealed brood among the three experimental groups. Sealed brood area was significantly different between formic acid-treated colonies and control colonies. Brood viability, adult bee population, returning foragers and honey production were not statistically different between menthol- and formic acid-treated colonies and control colonies although formic acid-treated colonies experienced, on average, lower honey production than either menthol-treated or control colonies. Queen behaviour patterns and the number of workers attending the queen in the retinue were not statistically different before versus after colonies were treated with formic acid.

I conclude that recommended, legal treatments of Apistan®, menthol, and formic acid are not detrimental to colony development or surplus honey production. The benefits gained from using formic acid to control parasitic bee mites far outweigh the slight decrease in sealed brood. Fluvalinate and menthol, when applied according to recommended methods, produce no adverse effects on honey bee colony development. Legal use of menthol and formic acid has no deleterious effects on surplus honey production.

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1.0 Introduction

The honey bee industry is an important component of agriculture world-wide. Canada is the fourth largest honey producing country in the world market and has the highest productivity of honey per hive in the world today (Winston, personal communication). In addition to products produced from apiculture, such as honey and beeswax, the most important aspect of beekeeping is pollination of agricultural crops. Over one-half billion dollars of Canadian crop production depend on pollination by honey bees (Scott-Dupree *et al.* 1995). Clearly, honey bee colonies are of significant agricultural and economic value in North America. Maintaining the health of honey bee colonies to obtain maximal productivity is thus of paramount importance.

More than 100 species of mites are associated with the honey bee, *Apis mellifera* L. (Grobov 1975). Of the many and varied diseases and parasites beekeepers are faced with, the two largest threats in North America are the parasitic mites, *Acarapis woodi* Rennie (tracheal mites) and *Varroa jacobsoni* Oudemans. Both mite species are capable of producing devastating effects on honey bee colonies (De Jong 1990; Eischen *et al.* 1989; Komeili and Ambrose 1989; Ball 1988; Beetsma *et al.* 1988; Drescher and Schneider 1988; Eischen 1987). *A. woodi* and *Varroa* have had a significant impact on beekeeping world-wide. Beekeepers are now forced to administer pesticides to their colonies, which is a practice new to many. There are a number of pesticides used to control *A. woodi* and *Varroa* throughout the beekeeping community. In Canada, fluvalinate, formulated as Apistan®, and formic acid are registered for *Varroa* mite control and formic acid and menthol are products registered for controlling *A. woodi* infestations.

Beekeepers are concerned about the advent of in-hive pesticide use and potential detrimental effects on their colonies. Fluvalinate, menthol, and formic acid, when applied according to recommendations, do not result in direct adult bee mortality. Sublethal colony effects resulting from acaricide use, however, may have a negative impact on colony development and honey production. Beekeepers must be informed of the impacts these chemicals have on their colonies. The increasing use and possible misuse of acaricides demands a thorough understanding of

negative effects on honey bee colonies resulting from acaricidal application. This study focused on whether sublethal effects in honey bee colonies arise from legal in-hive applications of the acaricides fluvalinate, menthol, and formic acid.

1.1 Effects of Mites on Honey Bees

1.1.1 Tracheal Mites

Acarapis woodi feeds and reproduces in the tracheal system of adult honey bees. Mite prevalence in honey bee colonies reaches a peak in late winter before declining in late spring to negligible levels in summer months (Otis *et al.* 1988; Scott-Dupree and Otis 1988). Moderate to heavily infested colonies (> 30% adult bees infested) experience significantly higher winter mortality than uninfested colonies or those with a low infestation rate (Otis and Scott-Dupree 1992; Bailey 1961; Bailey and Lee 1959; Bailey 1958), but summer or autumn mortality of infested colonies is seldom seen (Bailey and Lee 1959). Heavily infested colonies may survive, but their brood production is reduced (Otis and Scott-Dupree 1992; Wilson *et al.* 1990) and these colonies die earlier than non-infested colonies (Komeili and Ambrose 1989) presumably as a result of reduced longevity of adult bees (Maki *et al.* 1986; Bailey and Lee 1959; Bailey 1958). Adult worker bee longevity is reduced when bees are infested during pupal development (Schneider and Drescher 1987). Another study, however, observed no significant reduction in longevity as a result of *A. woodi* infestation (Gary and Page 1989). Colony strength, measured by adult bee population and the amount of brood present, and honey production decrease significantly in the presence of tracheal mites (Otis and Scott-Dupree 1992; Eischen *et al.* 1988; Eischen 1987). Lightly infested colonies (< 5%) produced, on average, 24.1 kilograms (kg) surplus honey while heavily infested colonies (86.7%) produced only 3.2 kg surplus honey (Eischen *et al.* 1989; Eischen and Dietz 1986). Wintering ability of tracheal mite infested colonies is a major concern to beekeepers in northern climates (Furgala *et al.* 1989; Otis *et al.* 1986). Colonies heavily infested with tracheal mites may abandon hives in addition to experiencing population decrease (Thoenes and Buchmann 1992). Tracheal mites also may contribute to death of infested colonies when combined with other diseases or poor weather conditions (BCMAFF 1992).

1.1.2 *Varroa* Mites

Varroa jacobsoni feeds on bee haemolymph. The mite feeds and reproduces on honey bee brood during the bees' late larval and pupal stages, inside the sealed cell. Female *Varroa* enter brood cells of larvae 170 hours old up to cell capping (Fuchs and Muller 1987) and lay eggs. Mite development and mating are completed before the bee emerges from the sealed cell. Only adult female *Varroa* survive to emerge with the adult bee; male and nymphal mites die when the cell is opened for bee emergence (Schulz 1984). Adult *Varroa* also live and feed on adult bees as a secondary food source prior to entering brood cells. *Varroa* weaken individual bees (De Jong *et al.* 1982) making it more difficult for an infested colony to maintain normal sanitation and environmental conditions in the hive which leaves colonies susceptible to other disease agents. The puncture of a bee's integument by the feeding mites allows invasion of viral, bacterial and fungal pathogens such as acute bee paralysis virus and *Melissococcus pluton*, the bacterium causing European foulbrood disease (Glinski and Jarosz 1992). Reduced body weight, misshapen wings, shortened abdomens (De Jong *et al.* 1982), reduced blood protein (Weinberg and Madel 1985) and reduced longevity (De Jong and De Jong 1983) have been attributed to *Varroa* parasitization of honey bees. All of these factors, alone or in combination, cause high mortality in *Varroa*-infested colonies (Bailey and Ball 1991; De Jong 1990). Colonies left untreated typically die within one or two years of infestation. *Varroa* infestation of three to seven percent in early spring significantly decreases honey production, and a seven percent spring infestation rate will kill colonies by fall (Gatien and Currie 1995).

1.2 Mite Distribution

1.2.1 Tracheal Mites

A. woodi is an endemic parasite of honey bees in Europe (Clark 1985). The first mites in North America were discovered in Mexico in 1980 (Wilson and Nunamaker 1982) and by 1984 they were detected in Texas. The first tracheal mites in Canada were discovered in Manitoba in 1986 (Anonymous 1986) and rapidly spread to other parts of the country (Dixon 1990). *A. woodi* is now found

throughout Canada, with the exceptions of Newfoundland, Vancouver Island, and the Gulf Islands of British Columbia (Winston, personal communication).

1.2.2 *Varroa* Mites

The original distribution of *Varroa* is related to that of its natural host, the Asian honey bee *Apis cerana*. *Varroa* spread to *A. mellifera* by the introduction of *A. mellifera* to areas also inhabited by *A. cerana* and subsequent movement of infested *A. mellifera* queens and colonies around the world (Clark 1985). *Varroa* causes no economic damage when cohabiting with *A. cerana* (De Jong *et al.* 1982), but has developed into a highly damaging parasite for *A. mellifera*. Currently, the only major beekeeping areas that are believed to be free of *Varroa* mites are Australia, New Zealand and Hawaii. *Varroa* was first identified in the United States in 1987 (Needham 1988) and is now widely distributed throughout the U.S.. In Canada, *Varroa* was first detected in southern B.C. in 1992. By fall 1993 the mites were found in most hives in the Fraser Valley of B.C. (Clark 1994). Currently, *Varroa* is found throughout B.C. except for Vancouver Island, the Gulf Islands and Powell River Regional District (Winston, personal communication). *Varroa* is now found in most beekeeping regions of Canada, although it has not yet reached high densities in parts of the prairie and maritime provinces.

1.3 Current Control Methods for *A. woodi* and *Varroa*

1.3.1 Tracheal Mites

Menthol

Menthol is scheduled for control of tracheal mites in Canada and the United States and was approved for use in Canada in April 1992 (Nelson *et al.* 1993). Menthol is an effective control method for tracheal mites when temperatures within the colony are high enough to adequately evaporate the menthol (Delaplane 1992; Cox *et al.* 1989; Moffett *et al.* 1989; Cox *et al.* 1988; Herbert *et al.* 1987). Spring treatments have been shown to significantly reduce mite populations (Duff and Furgala 1993, 1991). Cool conditions or fall treatments render menthol less effective for tracheal mite control (Moffett *et al.* 1989). Menthol crystals placed in the hive volatilize and the fumes kill the mites inside the bees' tracheae (Cox *et al.* 1986).

A number of menthol application methods have been described in beekeeping literature, but the menthol crystal packet, 50 g active ingredient (a.i.), is the most widely used form of menthol treatment that provides good mite control (Moffett *et al.* 1989). Application of the menthol-containing mesh bags on top frame bars appears to be a good method for cooler climates or if autumn treatment is planned (Herbert *et al.* 1988). The packets also may be placed on hive bottom boards (Cox *et al.* 1986). Packets can be purchased ready-made or fabricated by beekeepers (Duff and Furgala 1991; Herbert *et al.* 1987). Another less frequently used menthol application method is the use of menthol-impregnated foam strips hung from the top frame bars (Nelson *et al.* 1993; Nelson and Grant 1991).

Menthol 50 gram packets placed on hive bottom boards every two weeks for six weeks of continuous exposure resulted in 98% to 100% tracheal mite mortality (Cox *et al.* 1986). Tracheal mite prevalence in colonies treated with several different menthol applications was reduced to less than one percent (Nelson 1994; Nelson *et al.* 1993). Colonies treated with solid menthol cakes experienced 90% to 97% mite control (Cox *et al.* 1988).

Menthol is inconsistent in its effectiveness at tracheal mite control because its volatilization is temperature-dependent. Warm temperatures, minimum 20° C, are necessary to fully and efficiently vaporize the crystals (Cox *et al.* 1988; Herbert *et al.* 1988). Colonies should be placed in yards to maximize menthol vapourization in climates where spring temperatures may be cool. Other dispersal methods may be used to maximize vapourization in cooler climates such as menthol paste applied to cardboard or menthol-containing foam strips (Nelson *et al.* 1993; Herbert *et al.* 1988). Another alternative application method is to place cardboard squares that have been dipped in a menthol/vegetable oil mixture on the hive bottom boards (Nelson *et al.* 1993). The squares stay in place for 7-10 days with one or two treatments given to each hive. Menthol treatments must be initiated at least six weeks prior to the anticipated honey flow.

1.3.2 *Varroa* Mites

Fluvalinate

The most widely used treatment for *Varroa* control throughout North America is τ-fluvalinate (RS-α-cyano-3 phenoxybenzyl [R]-2-chloro-4-[trifluoromethyl] anilino-3-methylbutanoate), formulated as Apistan® strips. Fluvalinate belongs to the synthetic pyrethroid class of chemical insecticides/acaricides. The combination of favourable bee toxicity and acaricidal activity (Henderson 1988; Henrick *et al.* 1980) led to the development of fluvalinate, formulated in PVC resin (Apistan®) strips, to control *Varroa* mites. Fluvalinate-impregnated PVC plastic was approved for use in Canada in 1992 and 1988 in the United States. Apistan® strips are currently the only approved fluvalinate application method for *Varroa* control in North American honey bee colonies.

Fluvalinate is a nerve toxin that kills *Varroa* mites on contact. Dispersal of fluvalinate within the colony is through honey bee contact with the Apistan® strip. Fluvalinate is lipophilic; bees walk over the strips and the active ingredient adheres to the oils on the bees' body surface. Fluvalinate is then passed from bee to bee and finally from bee to the *Varroa* mite. Within hours of strip placement, all bees have come in contact with the compound. Adult mites contacting these bees will be killed by the acaricide. Ninety-nine to 100% *Varroa* control is achieved in five to six weeks of strip placement in colonies containing sealed brood (Zoecon 1989). The majority of mites on adult bees are killed within the first 24 hours of strip placement (Herbert *et al.* 1988). Apistan strips should remain in place for 42 days but no longer. Worker bees require 21 days to develop from egg to adult stage. Mites in capped brood cells escape contact with fluvalinate until they emerge with the adult bees. Placing Apistan strips in the hive for 42 days (two generations of worker bees) allows exposure of all adult mites to the acaricide. Maintaining the six week treatment period as outlined on the product label is essential for reducing the development of resistant mite populations while at the same time providing effective *Varroa* control. Spring treatments must be complete and strips removed prior to the first main nectar flow. Autumn treatments should be initiated following final honey

harvest (Zoecon 1989). Apistan® strips should be placed in hives when outside temperatures reach 12° C or higher.

Fluvalinate is a unique pyrethroid in that it is essentially non-toxic to honey bees with topical LD_{50}=18.4 μg/bee and oral LC_{50}=1000 ppm in nectar (Duff and Furgala 1992; Zoecon 1989; Henrick *et al.* 1980). Foliar fluvalinate residue is non-toxic and non-repellent to honey bees and does not inhibit plant pollination by bees (Estesen *et al.* 1992; Waller *et al.* 1988). At high rates of application, fluvalinate had the least impact on the odor learning response of honey bees of all pyrethroids tested (Taylor *et al.* 1987).

Investigations of Apistan® effects on honey bee colonies suggest there are no adverse effects on sealed brood area, honey production, queen acceptance, queen survival (Duff and Furgala 1992) or brood viability and queen supersedure rates (Pettis *et al.* 1991) when strips are used according to the manufacturer's recommendations. Honey bee queens treated with Apistan® Queen Tabs, one percent a.i., showed no abnormal mortality within the recommended three day treatment period (Pettis *et al.* 1991). Queen acceptance, supersedure and subsequent brood production were not adversely affected by queen exposure to Queen Tabs (Williams *et al.* 1994). Studies investigating fluvalinate's effectiveness against *Varroa* found that low concentrations of fluvalinate have negligible effects on honey bees in packages (Herbert *et al.* 1989; Witherell and Herbert 1988). *Varroa*-infested colonies treated with Apistan® experienced increased body weight of hive bees, maintenance of colony population size, and decreased incidence of misshapen newly emerged bees (Delaplane 1995).

Fluvalinate maintains its pesticidal activity at temperatures of 28° to 38° C (Henrick 1995). Retention of high acaricidal activity at high temperatures coupled with low honey bee toxicity make fluvalinate an excellent compound for control of parasitic mites in honey bee colonies where ambient temperatures may be as high as 37°C.

Formic Acid

Formic acid (65% concentration) was registered in Canada in 1992 for control of both *A. woodi* and *Varroa* mites. Formic acid, to a lesser degree than

fluvalinate, is an effective *Varroa* control (Apicultural Abstracts 1994; Clark 1994; Bolli *et al.* 1993; Bracey and Fischer 1989; Hoppe *et al.* 1989) and also is effective against tracheal mites, especially in cooler climatic conditions (Gatien and Currie 1995; Liu and Nasr 1992; Clark and Gates 1991; Hoppe *et al.* 1989). Formic acid has several advantages as an acaricide over menthol and fluvalinate. Formic acid is capable of controlling both *Varroa* and tracheal mites, is found naturally, at varying levels, in honey (Crane 1975; White 1975), is used as a preservative in some fruit products (Ritter 1981), and is less expensive than either menthol or Apistan®. One positive side effect of colony treatment with formic acid is the mortality of young wax moth larvae (Hoppe *et al.* 1989). Formic acid's pesticidal action has been observed in nature. Some birds groom themselves with the formic acid produced by ants, which is believed to help control ectoparasites (Ritter 1981).

Formic acid is an organic acid that acts as a cytotoxin to kill mites on contact (Gatien and Currie 1995). The acid fumes kill *A. woodi* in the tracheae of infested bees (Liu and Nasr 1992). In the case of *Varroa,* formic acid inhibits or arrests mite respiration (Bolli *et al.* 1993). The degree of mite control depends on the amount of acid evaporated within the hive over time (Befus-Nogel and Nelson 1994). Outside temperatures should be above 10° C for effective treatment with formic acid.

Several methods of applying formic acid to colonies have been developed including application of liquid formic acid (65% solution) to absorbent pads on top frame bars and direct application to the hive bottom board using a meter drench gun (Thomson, personal communication). Gel strips containing 30 or 60 grams of formic acid in a polymer gel and enclosed in a plastic wrapper with holes on the underside of the wrap are being tested. To date, gel strips have shown inconsistent evaporation rates. Recent advances in the application of formic acid involve the development of extended-release dispensing methods that not only provide increased safety to the applicator, but also reduce the number of trips necessary to complete the treatment, a major concern for commercial beekeepers working with large numbers of hives (Clark, personal communication). The new dispensing methods or formulations under investigation are being developed to achieve a high level of formic acid evaporation with minimal management manipulations. One

prolonged-release formulation, the German Illertisser Milben-Platten™, contains anhydrous formic acid on an absorbent paper pad enclosed in a sealed pouch applied weekly (three applications) to the top frame bars (Nelson 1994). A commercially available product, Mite Wipe™, is an absorbent pad that soaks up and holds a determined quantity of formic acid. The pads are replaced at four to 10 day intervals. A total of three applications are used for control of tracheal mites and five or six applications for *Varroa* control. Acid-soaked pads are a safer alternative to the drench gun for both bees and applicator. Formic acid treatments for *Varroa* control must cover a complete brood rearing cycle (if brood is present) to be effective (Bracey and Fischer 1989). Formic acid treatments must be complete at least 14 days prior to the start of the honey flow.

Tracheal mite levels in colonies treated with different formic acid application methods were reduced to between zero percent and 3.2% and these lower levels were significantly different from mite prevalence in control colonies (Nelson 1994). Clark and Gates (1992) obtained 92% tracheal mite control with spring formic acid treatment. Colonies treated with formic acid in the spring experienced significant *Varroa* mite mortality relative to untreated control colonies and infestation was reduced to almost zero after the final application. Results from the same study using formic acid to control *Varroa* found the chemical to be ineffective at infestation levels higher than 20% (Gatien and Currie 1995). Fall application of formic acid also reduced *Varroa* infestation levels (Gatien and Currie 1995). Colonies treated with formic acid experienced 94% control of *Varroa* population and 91% control of adult *A. woodi* (Hoppe *et al.* 1989). Numbers of dead adult tracheal mites were higher and numbers of eggs and nymphs were lower in formic acid-treated colonies than those in control colonies (Liu and Nasr 1992).

1.4 Potential Problems Associated with Acaricidal Compounds

Menthol

High ambient temperatures may be problematic for small colonies treated with menthol. Under these conditions increased brood and adult bee mortality were experienced (Cox *et al.* 1986).

Colonies overwintered with menthol foam strips showed slightly higher adult bee mortality than untreated colonies, although none of the differences between treated and untreated colonies were significant. Menthol treated groups in this experiment also had less brood and adult bees than control colonies (Nelson and Grant 1991).

Menthol can have a negative effect on queen emergence from sealed cells. Live queens emerged from 48% to 56% of cells placed in mating nucleus colonies treated with menthol crystal packets. The untreated group experienced 80% queen emergence. Reduced success of queen cells in menthol-treated colonies appeared to result from a failure to emerge or cell destruction by worker bees (Clark and Nelson 1989). Dead bee trap counts in a group of menthol-treated colonies were twice as high as counts in untreated colonies, although these results were thought to fall within a normal range for worker mortality (Cox *et al.* 1986).

Fluvalinate

Fluvalinate is so widely used that there is growing evidence that *Varroa* mites are developing resistance to this chemical (Lodesani 1995; Sugden 1995; Milani 1994). The synthetic pyrethroid class of chemical insecticides/acaricides has a history of inducing rapid resistance development in arthropod pest species (Henrick 1995). Improper or illegal use of Apistan® or other fluvalinate formulations may play a significant role in the development of mite resistance to fluvalinate. Evidence of mite resistance suggests the strong need for other mite control compounds and methods. Yearly or seasonal alternate use of formic acid and fluvalinate may reduce the rate at which mite resistance develops against either compound.

Formic Acid

Formic acid is corrosive and can damage human skin and eyes on contact. Inhaling formic acid fumes can also cause lung damage. Safe application of the acid must consider the health of both the applicator and the bees. High losses of bees can occur from acid spills in the hive. Formic acid can be dangerous to handle. Care must be taken with its use and adequate protective gear such as goggles, acid-resistant gloves and respirators must be worn by applicators. Spills of the liquid acid and mechanical breakdown of drench guns are common occurrences.

Formic acid can inhibit oxygen consumption of honey bee brood, and young larvae react with greater sensitivity to the acid than older larvae and young bees (Bolli *et al.* 1993).

Menthol, fluvalinate and formic acid may have the desired mortality effects on parasitic bee mites when used according to the recommended methods. However, extensive information on whether or not these compounds exert sublethal effects on honey bee colonies is not currently available. Other common insecticides can induce sublethal effects on honey bees. Diazinon use resulted in adverse effects on honey bee longevity and temporal division of labour tasks (MacKenzie and Winston 1989). Malathion and diazinon produced lethal and sublethal effects on worker honey bees (Smirle *et al.* 1984). Small colonies given parathion (0.1 ppm) or methyl parathion (0.02 ppm) reared less sealed brood, and experienced reduced honey bee survival and honey production (Barker and Waller 1978). Sublethal doses of parathion also prevented bees from communicating food source direction through the dance language (Schricker and Stephen 1970). Larvae exposed to carbofuran and dimethoate at concentrations sublethal to adult bees experienced depressed weight gain and died earlier than control larvae. Numbers of viable pupae also were reduced when pupal bees were exposed to the two chemicals at rates of 1.25 μg/g carbofuran or 0.313 μg/g dimethoate (Davis *et al.* 1988).

Currently, chemical acaricides must be used to achieve adequate control of tracheal and *Varroa* mite infestations and ensure profitable apicultural operations. Acaricides are now an integral feature of beekeeping in North America. There are some genetic strains of honey bees that demonstrate resistance to tracheal mites (Loper *et al.* 1992; Milne *et al.* 1991; Szabo *et al.* 1991; Gary and Page 1987), but this control measure has not been effective enough to date to eliminate the need for chemical treatments. Tracheal mite resistance in bee stocks may be accompanied by undesirable characteristics such as decreased resistance to diseases, decreased honey production or aggressive behaviour (Delaplane 1996). There is little evidence of resistance to *Varroa* mites in North American honey bee populations (Morse *et al.* 1991). Thus, North American beekeepers depend on acaricides placed inside live bee colonies for prolonged time periods. Because these pesticides are applied

directly into hives, there may be potential for sublethal effects on larval and/or adult honey bees. Beekeepers need to know what, if any, deleterious effects in-hive mite treatments have on their colonies, since sublethal effects on bees may cause adverse effects on colony productivity.

The purpose of this study was to determine whether exposure of adult and larval honey bees to acaricide concentrations not immediately lethal to adult bees resulted in any significant sublethal damage to colonies. The relationship between sublethal acaricide effects and honey bee colony productivity and honey production was the focus of this project. The experiments presented here measured numerous colony variables, including:

- worker mortality
- brood survival
- worker longevity
- sealed brood area
- foraging
- pollen load weight
- emerged bee weight
- attendance of queen by worker bees
- queen behaviours
- colony weight gain
- adult bee population
- honey production

to determine if three widely used acaricides, fluvalinate, menthol, and formic acid (two different application methods) produced sublethal effects in honey bee colonies, independent of mite presence.

2.0 MATERIALS AND METHODS

2.1 STANDARD HIVE EXPERIMENTS

Colony Conditions and Location: Spring/Summer 1995

Experimental colonies were located at Simon Fraser University, Burnaby, British Columbia, and studies conducted April-July, 1995. Thirty experimental colonies were initiated 25 April from two pound packages of bees from Vancouver Island, B.C. that were free of tracheal and *Varroa* mites. Colonies were sampled immediately for tracheal and *Varroa* mites to verify their mite-free status. Mites were not found in any of the colonies. All packages were queened with sister Carniolan queens of Australian origin. Packages were installed in standard Langstroth deep hive equipment. Colonies initially consisted of one 10-frame hive body with bees covering five frames at the time of colony initiation. All thirty colonies grew and required an additional hive body on 20 June to alleviate colony crowding.

Ten colonies were used in each of three treatments; control, Apistan®, and formic acid. One colony was dropped from the Apistan® treatment due to queen loss. Colonies were organized in two separate horseshoes to minimize worker drift between colonies, five pallets per horseshoe and three colonies per pallet. Each pallet housed a control, Apistan®, and formic acid colony. Location of colonies on the pallet was rotated counterclockwise to vary position and eliminate bias related to position and/or orientation effects. Hives faced south, east or west.

Dead bee traps (Pankiw 1991) were placed on colonies when the packages were installed in hives. Traps remained on colonies from the start of acaricide treatments and were removed three days after the final formic acid treatment.

All colonies were fed equal quantities of sugar syrup eight times and pollen patties twice throughout the experiment to stimulate colony growth.

Acaricides

Commercial formulations of the acaricides fluvalinate, formulated as Apistan®, and formic acid were used in the experiments. Apistan® is formulated as fluvalinate (10% a.i.) impregnated in polyvinyl chloride (PVC) strips. These strips are specially designed for use in honey bee colonies. A 65% liquid solution of formic

acid was used in the experiment, prepared by mixing a 90% stock concentrate with water by volume (Nelson 1994).

Treatments

Legal, recommended treatments of both acaricides were applied to colonies beginning on 3 May. Apistan® strips were suspended, one per colony (one per five frames of bees) in the centre of the brood chamber. Strips were removed 42 days after treatment began. Sticky boards for *Varroa* detection were placed in Apistan® colonies on the first day of the treatment period and removed the following day.

Formic acid (65%) treatments were applied to paper napkins on cardboard placed on the frame top bars. A total of four treatments of 15 ml formic acid were applied to each of the ten colonies in the treatment at four day intervals. The formic acid dose was applied to the absorbent boards using a metered drench gun. This formic acid treatment method is recommended for control of both tracheal and *Varroa* mites.

Observations

The following colony variables were measured to determine acaricide effects on colony development and productivity:

Colony Weight: Colony weight was determined by weighing hive bodies at the beginning and end of the experimental season. Empty hive bodies and frames were weighed on a platform scale before packages were installed. The empty weights were added to the two pounds (0.91 kg) of package bees to obtain initial colony weight. A tripod scale was used to weigh hive bodies and bees at the end of the experimental season.

Dead Bee Trap Counts: Numbers of dead bees recovered after package installation were determined by counting the dead bees in the trap for three days after colonies were initiated. Numbers of dead bees recovered after acaricide treatment were determined by collecting and counting the dead bees in the trap every day from 4 May to 18 May. Trap counts continued for three days past the final formic acid treatment on 15 May.

Traps were calibrated by adding 10 painted bees to each colony and counting the number of recovered painted bees in the traps the following two days. The traps had a dead bee recovery rate of 71± 4.6 %.

Brood Viability: Brood survival following acaricide treatment was determined by marking a patch of 100 cells containing eggs (Harbo and Szabo 1984) and uncapping and examining those cells 14 days later. Eggs reaching the late pupal stage were recorded as viable (Pettis *et al.* 1991). Compound eye colour was used as the aging characteristic for pupal bees. Pupal eye colour is unique to different stages of pupal development. As a result, the age of pupae can be judged to within one day (Jay 1962). Viability of brood was calculated as the number of pupae that developed from 100 eggs in a 10 x 10 cell area.

Longevity Counts: Groups of 100 newly emerged worker bees were tagged and reintroduced to their colony to determine worker longevity. Tagging occurred 22 days after acaricide treatments began. Emerged worker bees were obtained by removing emerging brood frames from their parent colonies and keeping the frames in emergence boxes in an incubator room (35° C) overnight to allow emergence from sealed pupal cells. Newly emerged workers were marked on their thoraces with coloured, numbered von Frisch tags and paint marks on their abdomens. Three tag colours were used to denote each treatment; white-control, yellow-Apistan®, green-formic acid. Six different paint colours were used to denote pallet number. An indelible pen mark on the tags was used to distinguish between colonies from different horseshoes. The marked worker bees were reintroduced to their colony within five hours of emergence and subsequent tagging.

Numbers of surviving marked bees were determined at six day intervals (beginning 31 May) by removal and inspection of each frame in each colony. All marked bees observed were recorded. Colony inspections continued until no tagged bees were located in any of the colonies. Final colony inspection took place 6 July. Marked individuals that drifted to other colonies were recorded but excluded from longevity data analysis.

Sealed Brood Area: The area of sealed (prepupae and pupae) brood in each colony was measured three times (24 May, 13 June and 6 July) in the experimental season.

Measurements were taken by placing a piece of clear Plexiglas™, with an inscribed 5x5 cm grid, over the sealed brood and counting the number of square centimeters of sealed brood on each side of the frame. Total number of covered grids were recorded and used to calculate the total sealed brood area for the colony.

Forager Counts: Numbers of pollen and non-pollen foragers were determined by monitoring the colony entrance and counting total returning foragers in a five minute period. Two hand-held counters were used to record pollen and non-pollen foragers separately. Three forager counts were conducted (29 May, 7 and 21 June) at four days, 13 days and 27 days, respectively, after worker bees were tagged. These dates corresponded with the peak foraging activity of those bees exposed to acaricides during adulthood (29 May) and peak foraging period for those workers exposed to acaricides during larval development (7 and 21 June).

Pollen Load Collection/Weighing: Pollen load weights were determined by collecting five pollen foraging workers per colony at the time of forager counts and immediately freezing those bees on dry ice. Pollen loads were later weighed on an electronic scale by weighing a bee with its pollen load, removing the pollen, weighing the bee again and taking the weight difference.

Emerged Bee Weight: Post-emergent bee weight was determined by collecting 10 newly emerged worker bees in vials and freezing them. The emerged workers were exposed to the treatments during their entire developmental period. The dead bees were later weighed on an electronic scale.

Data Analysis

All data were analyzed using the General Linear Model of SAS in an analysis of variance. Tukey's multiple means comparison was used to separate differences between treatment means (SAS Institute 1986). The data were analyzed for normality and heterogeneity of variance (SAS Institute 1987). Pre- and post-treatment dead bee trap count data, brood viability data and pollen load weight data were log-transformed prior to analysis to maintain heterogeneity of variance. Differences were accepted at the α=0.05 level.

Colony Conditions and Location: Spring/Summer 1996

Experimental colonies were located at two sites west of Dawson Creek, B.C. and studies conducted May-July, 1996. Thirty experimental colonies, 15 per apiary, were set up on 15 May from colonies that were overwintered in the Similkameen Valley of B.C.. Colonies were sampled for tracheal and *Varroa* mites to verify that mite levels were low in all experimental colonies.

Tracheal Mite Sampling:

Bees were collected in vials containing 70% ethanol. Thirty bees per colony were examined for presence of tracheal mites. Infestation was determined by removing the bee's head and pronotum, exposing the first pair of tracheal tubes and examining these tubes for presence of *A. woodi* under a dissecting microscope.

Varroa sampling:

Apistan* strips were placed in colonies and sticky boards added to the hive bottom board. Twenty four hours later, strips and boards were removed and the number of mites on each board was counted.

Mean tracheal mite infestation was 15.7 ± 4.3% and mean *Varroa* infestation was five ± 1.2 mites per colony. Suggested tolerable mite infestation levels are: 15% of bees in an apiary infested with tracheal mites (fall sample) and 100 *Varroa* mites per colony in spring (Clark, personal communication). According to these guidelines, the observed levels of tracheal and *Varroa* mites were not expected to affect the experimental results for the 1996 season. Although the tracheal mite level was approaching a level for concern, tracheal mite prevalence in colonies peaks in late winter and dwindles to negligible levels in summer (Otis *et al.* 1988). As a result, I felt that the infestation level detected in the experimental colonies was not high enough to confound treatment effects.

The two experimental apiaries were located 2.8 kilometers apart. Both apiaries were located adjacent to pasture and alfalfa crops.

Commercial colonies housed in standard Langstroth deep hive equipment were used in the study. Colonies were equalized prior to commencement of the experiment to consist of two hive bodies containing six frames of brood and enough

bees to cover the brood frames. All colonies received honey supers 24 June and 20 July.

Ten colonies were used in each of three treatment groups; control, formic acid and menthol. Treatments were split equally between each apiary with each yard containing five colonies of each treatment group. Colonies were organized four per pallet with each yard holding four pallets, one pallet in each corner of the apiary. Assignment of treatments to the colonies was completely randomized to eliminate bias related to position on the pallet and within the apiary.

Acaricides

Formic acid and menthol were the acaricides tested in the experiment. Formic acid used was a 65% liquid solution. A 40 ml quantity of the acid was applied to each Mite Wipe™ pad. Pads were soaked in acid overnight. The following morning, the pads had absorbed the measured quantity of acid and were transferred to a dry bucket for transport to the apiary. Menthol treatment consisted of a 20x20 cm piece of corrugated cardboard dipped in a menthol-vegetable oil mixture. Boards were kept frozen until used to preserve the potency of the active ingredient.

Treatments

Legal, recommended acaricide treatments were applied to colonies. Treatments began on 23 May. A total of five formic acid treatments were applied to colonies with four day intervals between each Mite Wipe™ application. Pads were placed on frame top bars. Two menthol treatments were applied. Menthol boards, one per colony, were introduced to the hive bottom entrance on 23 May. The second set of boards was placed in colonies eight days later.

Observations

Brood Viability: Brood viability was determined as described for 1995 experiment.

Sealed Brood Area Measure: Sealed brood was measured twice during the experiment, prior to acaricide treatment and during the treatment period. Measurement methodology was as described for 1995 experiment.

Adult Bee Population: Adult population in each colony was determined twice during the experiment, prior to acaricide treatment and during the treatment period. Adult population was measured using a Plexiglas™ sheet inscribed with a 5x5 cm grid placed over each frame on which bees were present. Total number of covered grids

was recorded and used to calculate the total adult bee population. A value of 1.5188 bees/cm^2 was used to estimate the total adult bee population in a hive (modified from Burgett and Burikam 1985).

Forager Counts: Numbers of pollen and non-pollen foragers were determined by monitoring the colony entrance for two minutes and counting returning foragers in that period. Two hand-held counters were used to record pollen and non-pollen foragers separately. Three forager counts were conducted, prior to acaricide treatment, during treatment and post-treatment.

Honey Production: Honey production was determined by weighing 30 empty honey supers and calculating the mean weight, 9.5 kg ± 0.13 kg. Full supers were removed from colonies and weighed 14 August. The difference between the full and mean empty super weight was calculated and this value was used as total honey production for the colony.

Data Analysis

All data were analyzed using the General Linear Model of SAS in an analysis of variance. Tukey's multiple means comparison was used to separate differences between treatment means (SAS Institute 1986). The data were analyzed for normality and heterogeneity of variance (SAS Institute 1987). Brood viability data were log-transformed to maintain heterogeneity of variance. Differences were accepted at the α=0.05 level.

2.2 OBSERVATION HIVE EXPERIMENT

Colony Initiation and Location

Experimental hives were located at Simon Fraser University, Burnaby, B.C.. Five four-frame observation hives made of Plexiglas™ were used in the experiment. Three frames of bees were used in each hive. The top frame space was left empty to facilitate introduction of formic acid-soaked towels to the hives. All colonies were of equal population. Each queen was given a paint mark on her thorax for ease of location and tracking in the hive.

Acaricide

A 65% formic acid solution was used in the experiment.

Treatment

Individual rolled paper towels were soaked with 10 ml of formic acid (65%). The formic acid treatments were measured in a small beaker and applied to the paper towel in a bucket. The towels were saturated with acid but not dripping. The rolls were introduced to hives via semi-circular portals located near the top of the hives.

Observations

Three trials of the experiment were conducted 17, 19 and 21 July, 1995. Queen behaviours were observed and recorded one hour prior to and one hour after formic acid introduction. Observation periods were 10 minutes in length. Three queen behaviours were observed and recorded: egg laying, stationary, and walking. The length of time the queen spent engaged in each behaviour during the ten minute observation period was recorded. The number of workers in the retinue was recorded before and after formic acid treatment. Outside temperature was recorded during each of the trials.

Data Analysis

Number of workers in the retinue data were analyzed for normality and heterogeneity of variance. Data were square root transformed to stabilize variance and subsequently analyzed by the General Linear Model of SAS in an analysis of variance. Tukey's multiple means comparison was used to separate differences in the mean number of workers in the retinue before and after formic acid treatment.

between the experimental colonies (SAS Institute 1986). Queen behaviour data before and after formic acid introduction, were analyzed by constructing a ternary plot representing the length of time the queen spent in each activity in the observation period. Differences were accepted at the α=0.05 level.

3.0 RESULTS

1995 Experiments: Standard Hive Experiment

Colony Weight Gain: Total colony weight gain among the three groups, control (13.2 kg ± 1.0), Apistan® (10.9 kg ± 1.1) and formic acid (13.6 kg ± 1.0), was not statistically different (P > 0.05) over the experimental season. Comparison of colony weights prior to commencement of acaricide treatments revealed no statistical differences (P > 0.05) between the treatment and control groups.

Dead Bee Trap Counts:

Before Treatment

The number of adult bees recovered from dead bee traps prior to acaricide treatment was not statistically different (P > 0.05) among control, Apistan®- and formic acid-treated colonies (Fig. 1).

During / After Treatment

The number of adult bees recovered from dead bee traps during and after acaricide treatment was not statistically different (P > 0.05) among control, Apistan® and formic acid groups (Fig. 2).

Brood Viability: Brood viability, the number of eggs that survived to become viable pupae, was not statistically different (P > 0.05) among control and acaricide-treated groups (Fig. 3).

Worker Longevity: Worker longevity was not statistically different between Apistan®- and formic acid-treated colonies and control colonies (P > 0.05) (Fig. 4). Longevity was lowest in the formic acid treated colonies (23.8 days ± 0.6) and highest in Apistan® treated colonies (24.5 days ± 0.7). Worker longevity in the control group was 24.2 days ± 0.6.

Sealed Brood Area: Total combined sealed brood area was not statistically different among the control and acaricide treatment groups (Fig. 5), nor was there a difference among control and treatment groups when each assessment day was analyzed individually (P > 0.05).

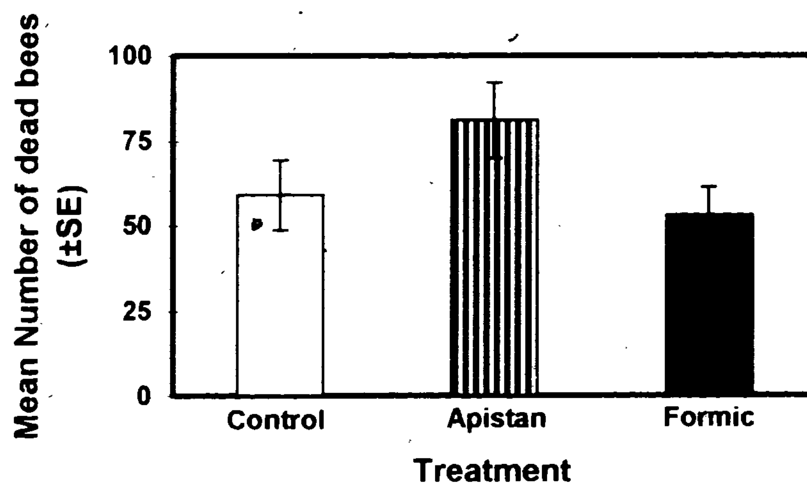

Figure 1. Mean total number of dead bees (±SE) recovered from dead bee traps before acaricide treatment.

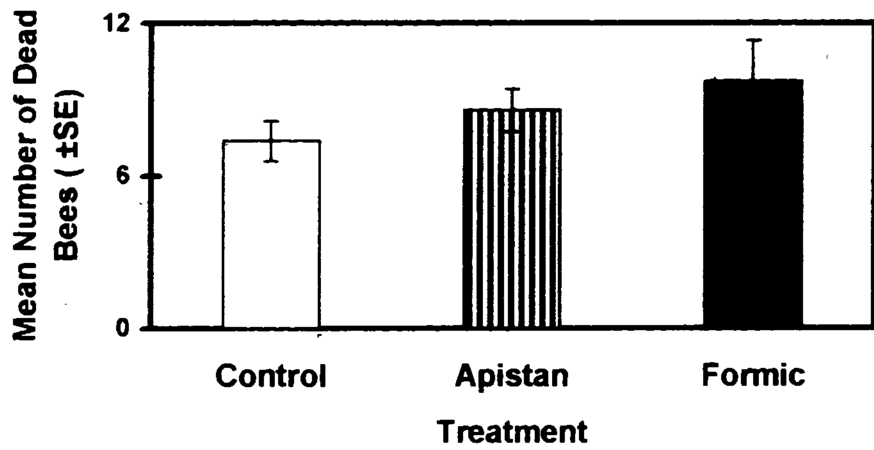

Figure 2. Mean total number of dead bees (±SE) recovered from dead bee traps, during and after acaricide treatment.

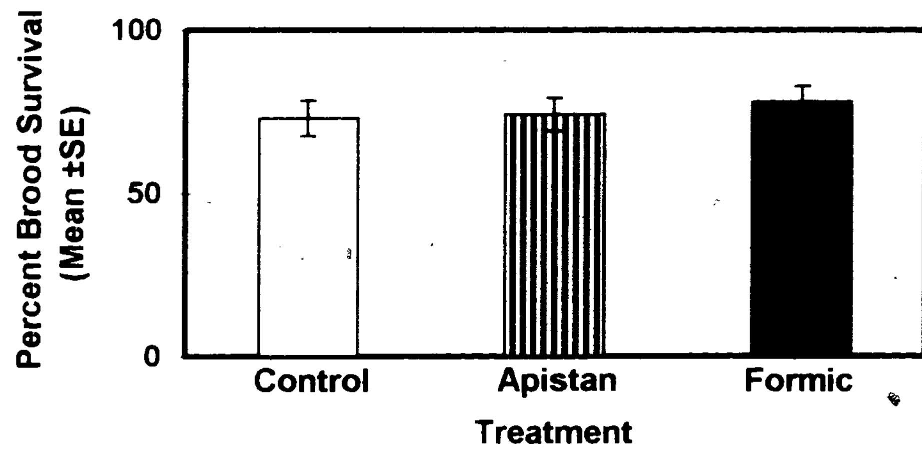

Figure 3. Percentage of eggs (Mean±SE) that survived to become viable pupae during acaricide treatment period.

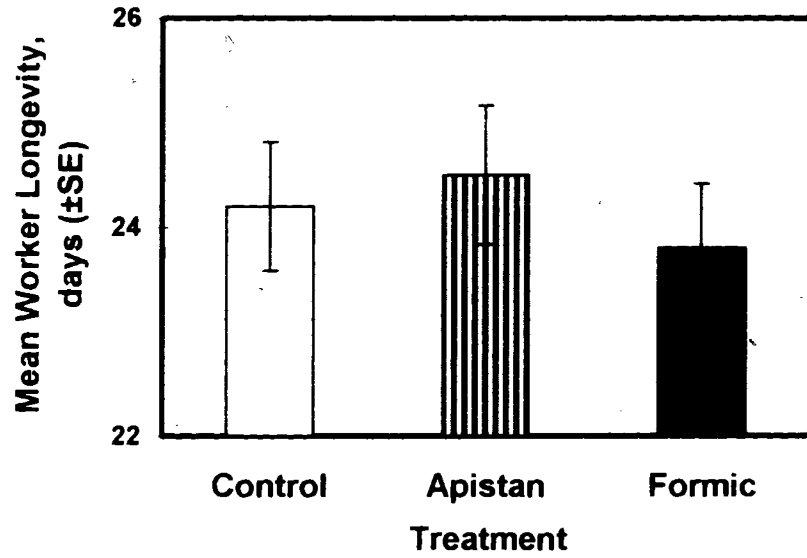

Figure 4. Mean number of days (±SE) worker bees survived after being exposed to acaricides during their developmental period.

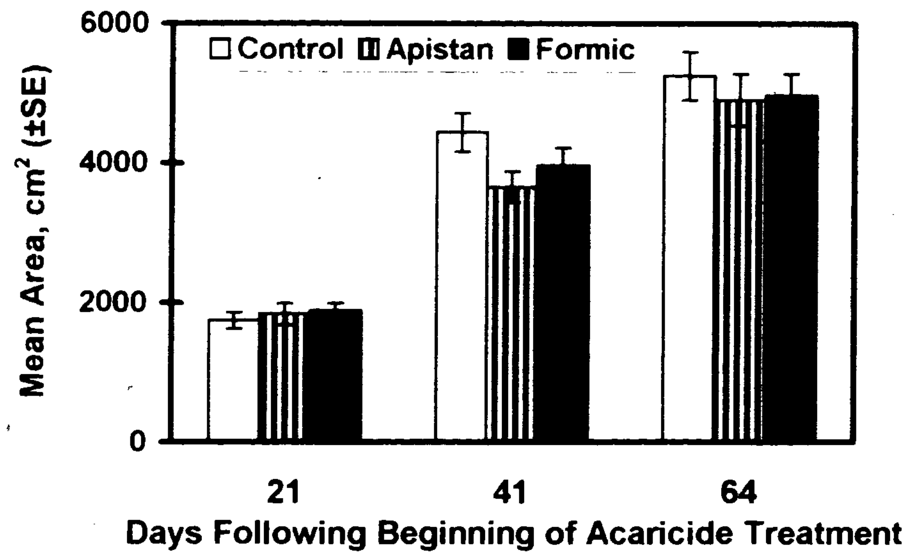

Figure 5. Mean sealed brood area (±SE) in control and acaricide-treated colonies measured on three assessment days during the experiment.

Returning Foragers - Total, Pollen, Non-pollen: The mean combined number of foraging workers, pollen and non-pollen foragers, returning to the hive was not different among the three treatment groups (P > 0.05), control (150 bees ± 10), Apistan® (148 bees ± 11), and formic acid (155 bees ± 10) (Fig. 6). Analysis of the number of returning pollen or non-pollen foragers revealed no statistical differences among control and treatment groups (P > 0.05) and there were no statistically significant differences (P > 0.05) when each assessment day was analyzed individually.

Pollen Load Weight: Total pollen load weight was not statistically different (P > 0.05) among the control, Apistan®, or formic acid treatment groups (Fig. 7).

Emerged Bee Weight: Mean weight of post-emergent bees was not statistically different (P > 0.05) among the three groups, control (105 mg ± 1.6), Apistan® (105 mg ± 1.8), and formic acid (110 mg ± 1.7).

1995 Experiments: Observation Hive Experiment

Workers in Retinue: Analysis of the number of bees in the retinue indicated no significant differences between numbers of bees attending the queen (P > 0.05) before versus after formic acid introduction to the observation hive (Fig. 8).

Queen Behaviour: A plot illustrating queen behaviour patterns before versus after formic acid introduction revealed no consistent changes in queen activities. The lack of a trend in behavioural patterns indicates there was no statistical difference in the amount of time queens spent in each activity before versus after formic acid introduction to the hive (Fig. 9). One colony in the experiment was not included in the final analysis because the queen was balled by workers after the formic acid was introduced to the colony.

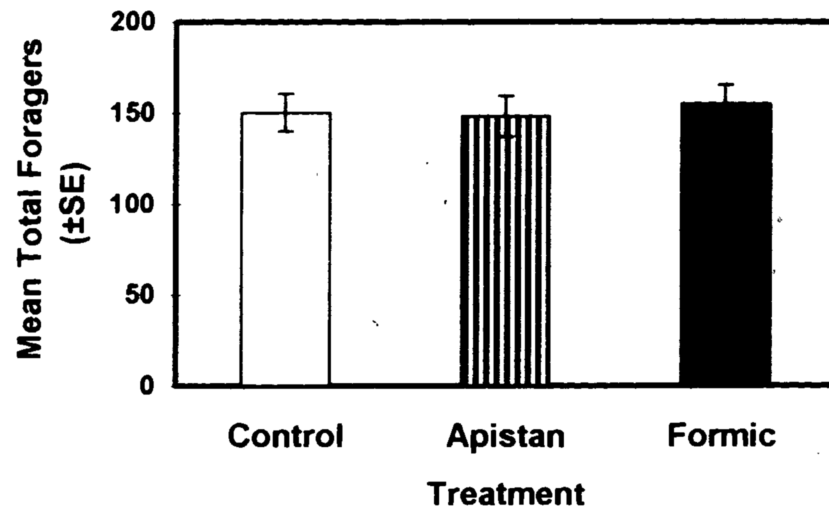

Figure 6. Mean combined number of pollen and non-pollen foragers (±SE) returning to control and acaricide-treated colonies in five minute observation period.

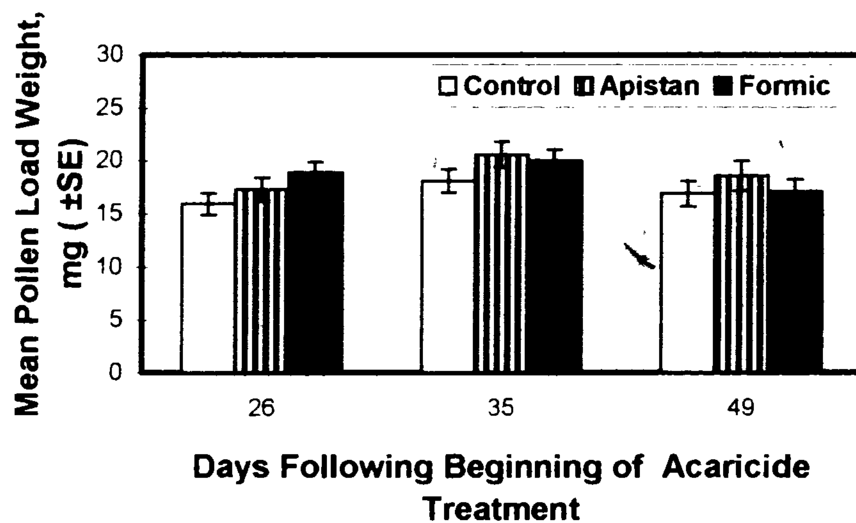

Figure 7. Mean pollen load weight (±SE), removed from corbiculae of five pollen foraging worker bees per colony and measured on three days during the experiment.

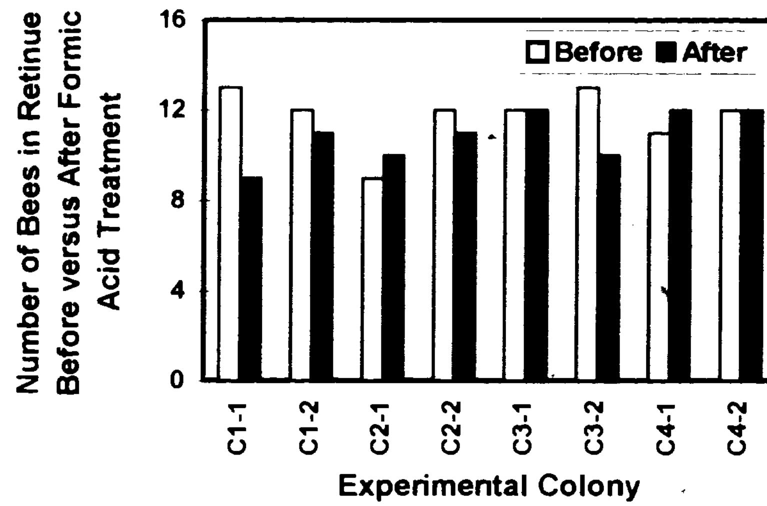

Figure 8. The number of worker bees in the retinue before versus after formic acid introduction to observation hives (C1-1 indicates colony number followed by trial number).

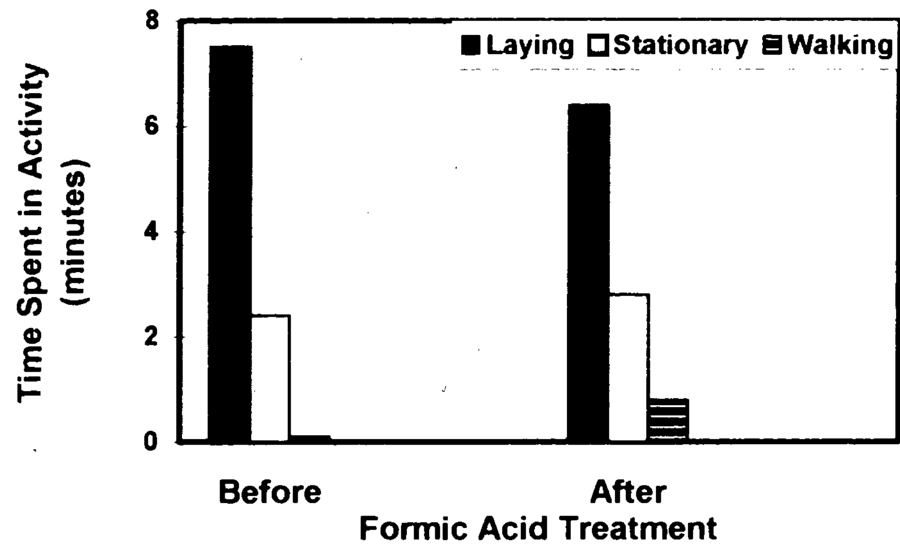

Figure 9. Amount of time during a ten minute observation period the queen spent engaged in one of three different activities, egg laying, stationary, or walking, before versus after formic acid treatment.

1996 Experiment

Brood Viability: Brood viability was not statistically different ($P > 0.05$) between control, menthol- and formic acid-treated colonies (Fig. 10).

Sealed Brood Area: Sealed brood area, measured prior to commencement of acaricide treatment, was not statistically different ($P > 0.05$) among control (3028 $cm^2 \pm 220$), menthol (2983 $cm^2 \pm 220$) and formic acid (2710 $cm^2 \pm 220$) groups. Sealed brood area of formic acid-treated colonies was statistically smaller than sealed brood area in control colonies ($P=0.05$) during the acaricide treatment period (Fig. 11).

Adult Bee Population: Total adult bee population was not statistically different ($P > 0.05$) among the three groups either before or during the acaricide treatment period (Fig. 12).

Returning Foragers - Total, Pollen, Non-pollen: The total number of foraging workers, pollen and non-pollen foragers, returning to the hive was not statistically different among the three groups ($P > 0.05$), control (126 bees $\pm$ 8.9), menthol (126 bees $\pm$ 8.9) and formic acid (113 bees $\pm$ 8.9) (Fig. 13). Analysis of the combined number of returning pollen or non-pollen foragers revealed no significant differences among the three groups ($P > 0.05$) and there were no differences between control, menthol- and formic acid-treated colonies when each assessment day was analyzed individually ($P > 0.05$).

Honey Production: Colony honey production among control, menthol- and formic acid-treated colonies was not statistically different (Fig. 14), although formic acid-treated colonies produced, on average, less honey (34.4 kg $\pm$ 7.5) than menthol-treated (43.9 kg $\pm$ 7.5) or control colonies (41.5 kg $\pm$ 7.5).

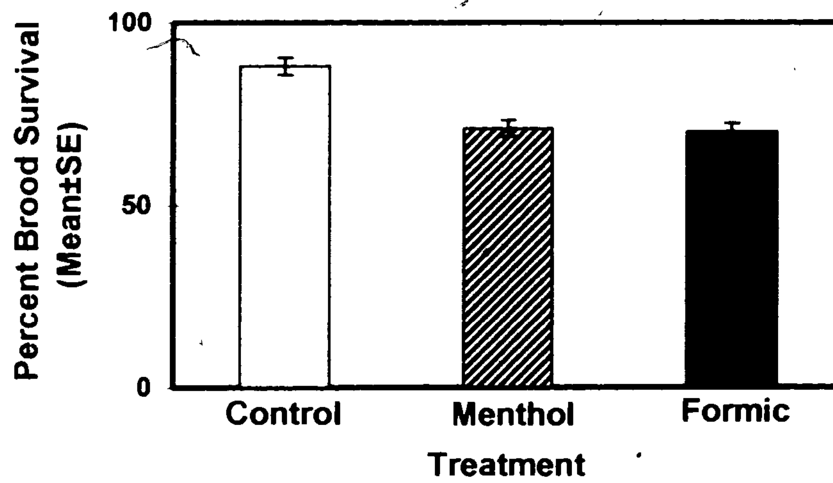

Figure 10. Percentage of eggs and young larvae (Mean±SE) that survived to become viable pupae during acaricide treatment period.

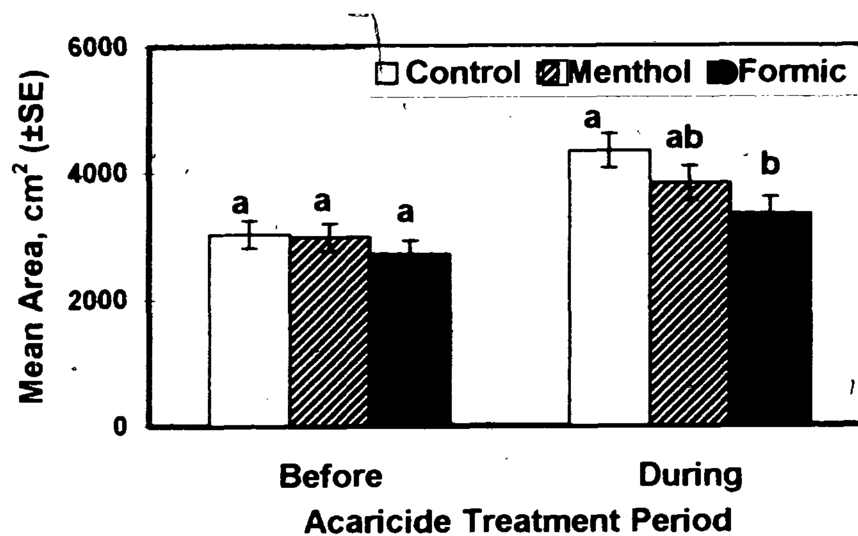

Figure 11. Mean sealed brood area (±SE) in control and acaricide-treated colonies measured on two assessment days during the experiment. Within a treatment period, bars with different letters are statistically different ($P < 0.05$).

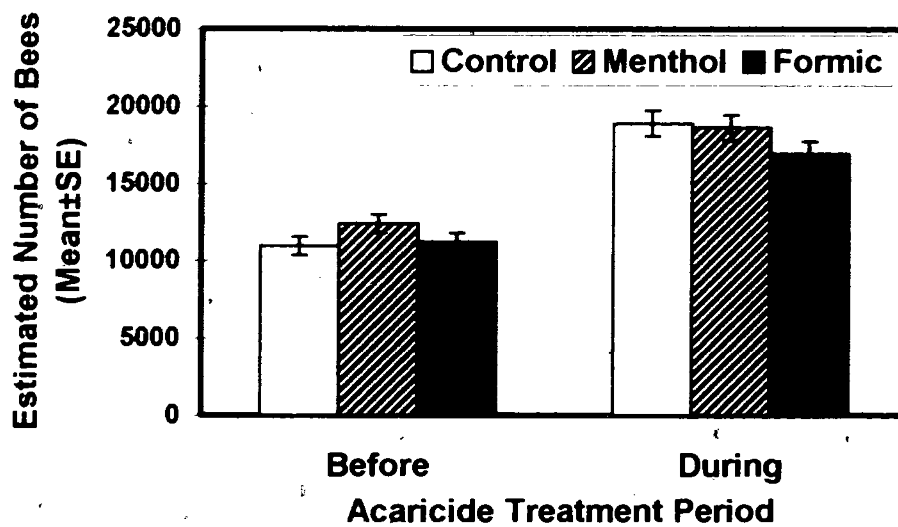

Figure 12. Mean total adult bee population (±SE) in control and acaricide-treated colonies measured on two assessment days.

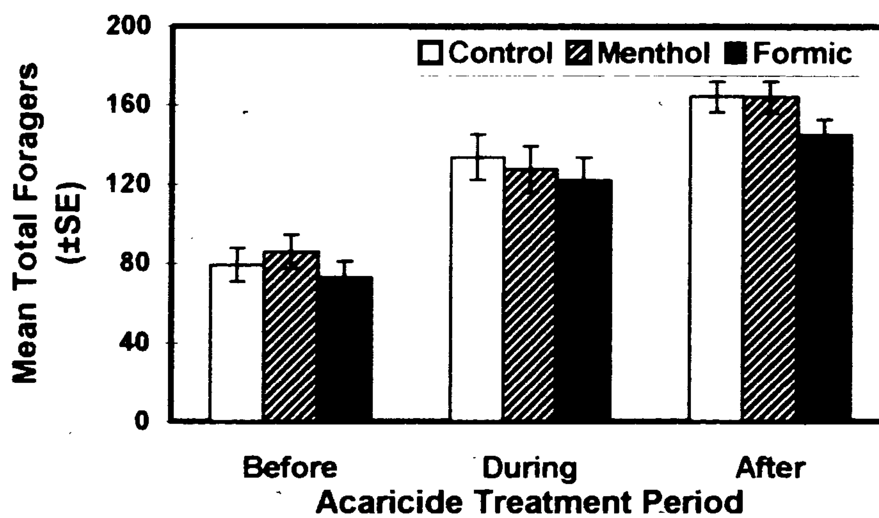

Figure 13. Mean number of pollen and non-pollen foragers (±SE) returning to colonies in two minute observation period on three assessment days during the experiment.

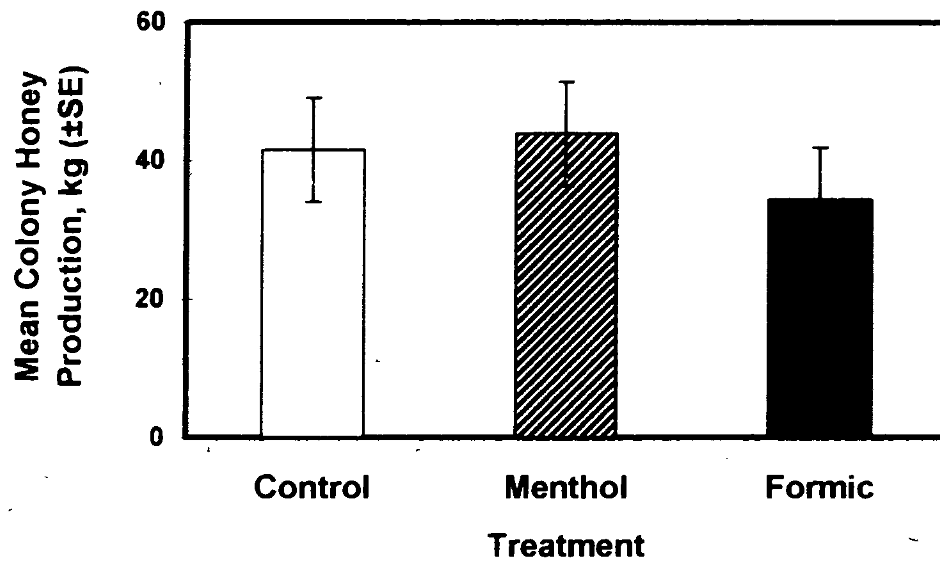

Figure 14. Mean surplus honey (±SE) produced by control and acaricide-treated colonies.

4.0 DISCUSSION

The results of my study indicate fluvalinate, formulated as Apistan® strips, and menthol, applied as cardboard squares dipped in a menthol-vegetable oil mixture, induced no adverse effects on individual honey bee workers, colony health, or colony productivity. Formic acid applied to Mite Wipe™ pads adversely affected brood rearing, although not enough to influence honey production.

Apistan®

Measurements of colony weight gain, adult bee mortality, brood viability, worker bee longevity, sealed brood area, foraging activity, pollen load weight and post-emergent bee weight revealed that Apistan®, when used according to the manufacturer's recommendations, poses no threat to a colony's overall development, health and related productivity. These results are consistent with the literature pertaining to Apistan® and its effects on honey bees. The relatively low toxicity of fluvalinate to honey bees has been reported by several studies (Duff and Furgala 1992; Zoecon 1989; Waller *et al.* 1988; Taylor *et al.* 1987; Stoner *et al.* 1984; Henrick *et al.* 1980). Pettis *et al.* (1991) demonstrated that mite-free worker bees exposed to Apistan® strips (2.5 % a.i.) did not experience a subsequent increase in mortality. In the same study, brood viability was not different between mite-free queen bees exposed to Apistan® Queen Tabs (1% a.i. concentration) and those not exposed. Investigations of Apistan® used in *Varroa*-infested colonies indicated the product resulted in favourable or neutral colony effects (Delaplane 1995).

In acaricide-treated and control colonies, worker bee longevity was highest in Apistan®-treated colonies. It is possible that workers in Apistan®-treated colonies experienced extended worker longevity because the infestation of *Varroa* mites (all colonies had at least a few *Varroa* present at the end of the experiment) was suppressed for a longer period in those colonies than the formic acid or control colonies. Perhaps the extended mite-free period in Apistan® colonies conferred a health advantage to the workers in those colonies, resulting in increased worker longevity.

It appears, from my research and other previously conducted studies, that Apistan®, when applied according to the manufacturer's recommendations, is safe to use in honey bee colonies and does not result in deleterious effects on colony health, development and/or productivity.

Menthol

Menthol treatments administered in our study did not produce adverse effects on honey bee brood viability or colony development (measured by sealed brood area). In other studies, menthol treatments resulted in short-lived negative effects on adult bee and brood mortality and had a repellent effect on adult bees (Duff and Furgala 1992; Cox *et al.* 1989; Wilson *et al.* 1988; Cox *et al.* 1987,1986). However, other research was not in agreement with those findings (Duff and Furgala 1991; Wilson *et al.* 1990). Duff and Furgala (1992) found that menthol application to newly assembled division colonies significantly reduced brood area in these small colonies and suppressed upward expansion of the brood nest during the colonies' first year. Experimental results from one study found no significant differences in colony development between menthol-treated colonies and untreated colonies, but, menthol-treated colonies experienced abnormal brood rearing behaviour, and normal practices resumed only after the menthol was removed (Nelson *et al.* 1993). Some evidence of suppressed brood rearing was detected as a result of menthol foam strip and dipped cardboard treatments, but, sealed brood production in these colonies was not significantly different among the menthol-treated or control colonies (Nelson 1994).

Menthol's acaricidal activity is highly temperature dependent; 20° C is considered the minimum temperature for volatilization (Wilson *et al.* 1990; Moffett *et al.* 1989; Cox *et al.* 1988; Herbert *et al.* 1987). Daytime temperatures in my study area generally fell below 20° C during the menthol treatment period. These cooler than normal temperatures may have had an effect on the volatilization rate of menthol in the hives. The lack of noticeable negative effects on brood may be due in part to a decreased release of menthol within the hives. In areas that experience a cool spring climate it may be more beneficial for mite control to place the menthol treatment on frame top bars over the cluster of bees rather than on the bottom

board. Placing menthol above the cluster would utilize heat generated by the bees and aid in evaporation of the menthol. Menthol vapours are heavier than air, so placement of the treatment at the top of the hive would ensure better dispersal of the vapours throughout the colony.

Honey production of menthol-treated colonies in my experiment was not significantly different from formic acid-treated or control colonies. In fact, of the three experimental groups menthol-treated colonies produced, on average, the highest honey yield for the 1996 season. The lack of any significant differences in foraging behaviour between the three groups in the experiment lends further support for the absence of any negative effects on colony honey production. A study by Duff and Furgala (1992) found menthol application, 50 gram a.i. packets, did not adversely affect net honey production during seasons of high nectar flow. In other studies, honey production was lower in menthol-treated colonies particularly when foam strip and dipped cardboard applications were used. Significant differences between treated and control colonies were evident in those studies (Nelson 1994; Nelson *et al.* 1993). It should be noted, however, that high mite levels also reduce colony honey production (Eischen *et al.* 1989; Eischen and Dietz 1986).

<u>Formic Acid</u>

Colony development, as measured by the area of sealed brood, revealed no differences between control, Apistan®- or formic acid-treated colonies in the 1995 experiment. Results from 1996 indicated sealed brood area was lowest in formic acid-treated colonies, and this difference between formic acid and control colonies was significant. Nelson (1994) observed no significant differences in sealed brood area between control colonies and those exposed to four different formic acid application methods. Hoppe *et al.* (1989) felt formic acid damage to eggs and young larvae was possible, but the brood loss appeared immediately following formic acid application suggesting this slight decrease could be tolerated because it had little influence on the total colony population. Results from my research indicated formic acid induced some brood loss during the treatment period, which was confined to brood directly adjacent to the formic acid-soaked pads. The lack of significant difference in honey production among the three groups in the experiment indicated the reduced brood production did not have a great impact on colony

development or productivity. However, of the three groups in the experiment, formic acid colonies produced, on average, the lowest quantity of surplus honey. The reduction in brood experienced in 1996 might suggest some caution in using formic acid under Peace River conditions, but the lack of observed differences in colony productivity between formic acid and control colonies suggests the negative impact of formic acid on brood is short-lived and not damaging enough to warrant the discontinuation of formic acid use.

In my study, worker bee longevity was lowest in formic acid-treated colonies, but this difference from control and Apistan®-treated colonies was not statistically significant. Worker bees tend to be short-lived in summer months in temperate climates with mean longevity of 15-38 days (Winston 1987; Winston *et al.* 1983; Winston *et al.* 1981; Michener 1974). Although formic acid-treated colonies experienced lower worker longevity than Apistan®-treated or control colonies, their life-span was well within the observed normal range. Fumigation of honey bee colonies with formic acid for 21 consecutive days resulted in no negative effects on worker bee longevity (Garg *et al.* 1984).

Adult bee mortality did not increase significantly following formic acid application to colonies. Although formic acid-treated colonies experienced the highest adult bee mortality of the three study groups in 1995, this observation may have been attributable to lack of care in application of formic acid to absorbent pads, because liquid formic acid dribbled on bees can cause extensive bee mortality. My findings are in keeping with results of Hoppe *et al.* (1989) who observed no increase in bee mortality following formic acid treatments. Nelson (1994), however, found the total adult mortality for liquid formic acid treatment to be eight times higher than the total count in control colonies and this difference was significant. Many of the highest counts in Nelson's study were observed the day following formic acid application which would seem to indicate the treatment caused the increased adult mortality.

Most beekeepers attempt to maximize the amount of honey their colonies produce. Although formic acid-treated colonies in our study produced, on average, less honey than menthol-treated or control colonies, this difference was not

statistically significant. Poor weather conditions in the study area in 1996 resulted in low overall honey yields. During periods of poor weather, bees are confined to the hive which increases their exposure to in-hive acaricide treatments. Under such conditions, any adverse effects resulting from the acaricide treatments would be amplified. In other studies, honey production was not significantly different among control colonies and groups of colonies receiving different formic acid treatments (Liu and Nasr 1992). However, all treated groups in another experiment experienced lower honey production than the control colonies (Nelson 1994).

The balling of a queen by worker bees and her subsequent disappearance was noted after formic acid was introduced to an observation hive. Other incidents of queen loss following formic acid application have been acknowledged by other researchers and beekeepers. Nasr (personal communication) found that use of 85% formic acid (which is not currently approved for use in Canadian colonies) resulted in bees balling and killing their queen. Other anecdotal information (see Wilson *et al.* 1993) has implicated formic acid application in queen loss events, but it appears this phenomenon does not occur frequently, nor is it easily reproduced or well-documented. Perhaps a specific set of requirements, both environmental and within the colony, must be met before queens are killed by their workers.

Formic acid poses serious health concerns for both applicators and honey bees if it is not handled and administered with care. It is highly corrosive and the fumes are capable of damaging vertebrate lungs. Fortunately, precautions such as wearing protective gloves, goggles and respirators allow beekeepers to safely apply formic acid to their colonies. Development of safer, easier to use formulations and application methods are helping to reduce the number of bees lost as a result of formic acid treatments. Formic acid use in honey bee colonies has been implicated in brood reduction, increased adult bee mortality, reduced honey production and queen loss, however, these effects are equivocal because other research with formic acid does not always result in the same, negative outcomes. There are many factors other than formic acid that could contribute to the adverse colony effects listed above. Colony health, mite infestations, disease, environmental conditions and queen vigor are factors that could act in concert with formic acid to influence colony health, development and honey production. Formic acid is very valuable for

Varroa mite control because it is a viable alternative to Apistan®. Mite resistance to fluvalinate is already a concern. Implementing an integrated pest management strategy where formic acid treatment is alternated with Apistan® may allow beekeepers to circumvent the mite's resistance mechanisms. Formic acid is effective against both *Varroa* and tracheal mites which further adds to its important role in beekeeping.

Considering the positive and negative aspects of formic acid use in honey bee colonies, it appears that formic acid's beneficial characteristics outweigh its potential problems as long as care is taken when handling or applying the chemical. It is imperative that beekeepers use only the recommended treatment methodology or serious physiological damage to bees and applicator may result.

5.0 CONCLUSION

This study examined the effects of fluvalinate, formulated as Apistan[x] strips, menthol, and formic acid on the development, health and productivity of colonies exposed to these three acaricides. The findings of my experiments provide further evidence of the effects that these widely used compounds have on the well-being of honey bee colonies.

There was a significant effect of formic acid use on the amount of sealed brood in the colony. Formic acid colonies had lower sealed brood area than control colonies. There were no detrimental effects of formic acid on worker bee longevity, worker foraging behaviour, pollen load weight or colony honey production. Furthermore, formic acid had no negative effects on queen behaviour or the number of worker bees attending the queen in the retinue. However, one event of a queen being balled by worker bees was observed following introduction of formic acid to the observation hive.

Apistan[x] strips, when applied according to the manufacturer's recommendations, appear to be safe to honey bees and resulted in no adverse effects on colony health or development. Menthol, administered as cardboard squares dipped in a menthol-vegetable oil mixture, produced no negative effects on colony development or subsequent honey production.

Adult and larval honey bees were exposed to concentrations of acaricides not immediately lethal to adult bees. The concentrations used in my experiments were those recommended by manufacturers and the apiculture community. Exposure of bees to these acaricide concentrations resulted in only one significant sublethal effect on colonies, sealed brood production. This significant result was associated with formic acid application. Other research found that detrimental effects of formic acid on brood occur directly after application and resulted in short-lived brood reduction. My results are in agreement with these findings. Although decreased brood was significant in my study, the negative effects are not damaging enough to warrant discontinuation of formic acid for control of the parasitic honey bee mites, *Acarapis woodi* and *Varroa jacobsoni*. However, some improvements in

formulation and application methodology would be justified to reduce the negative effect on brood production.

The results of my study help to emphasize the importance of legal, recommended administration of chemical acaricides to honey bee colonies. Legal, properly applied acaricide treatments result in few deleterious individual bee or colony effects. There is much anecdotal information on non-sanctioned mite treatments used by beekeepers throughout the world. Fluvalinate is available in formulations other than Apistan® strips for agricultural use. These other formulations are attractive to beekeepers because the cost per treatment is lower than Apistan® treatments. However, beekeepers are compromising their ability to control *Varroa* mites every time they devise and use their own fluvalinate treatments. Arthropods can develop resistance to pyrethroid insecticides/acaricides and there has been speculation that illegal use of fluvalinate, especially in Europe, has induced rapid development of resistant *Varroa* mite populations on that continent. North America cannot be far behind in this respect.

There are many homemade mite treatments using 65% formic acid in unsubstantiated methods; other remedies advocate use of 85% formic acid. In Canada, 65% formic acid is registered for use in beekeeping and only those application methods tested and approved by the apiculture community should be used. Formic acid is capable of causing physiological damage to both bees and applicators. Care and thought must be given to formic acid application or the health of both bees and beekeepers will be compromised.

Menthol treatments for tracheal mites are effective, but very temperature dependent. Inappropriate application conditions such as excessive heat causing rapid melting or volatilization of menthol can have a negative impact on colony brood production. Menthol use may be made more efficient through development of better formulations or application methods. The low incidence of deleterious effects on honey bees when fluvalinate, menthol, and formic acid are used according to recommendations underlines the value of these chemicals in the control of parasitic bee mites. Alternating use of acaricides is important for discouraging resistance in mite populations. The appropriate and alternate use of these chemicals ensures that their efficacy against mites will be maintained, while beekeepers can also rest

assured that the acaricides are not compromising the development and subsequent productivity of their colonies. Until viable strains of mite-resistant bees are developed, beekeepers are highly dependent upon chemical acaricides to maintain the health and productivity of their colonies. The findings of my study may help those in the beekeeping industry feel more comfortable when applying these chemicals to honey bee colonies.

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