In-Hive Miticides and their Effect on Queen Supersedure and Colony Growth in the Honey Bee (Apis mellifera)

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Abstract

Honey bees (Apis mellifera) contribute an estimated $200 billion annually to the global economy, primarily through crop pollination. Despite their importance, the number of managed honey bee colonies continues to decline. Recent surveys have shown that colony losses are attributed in great part to problems associated with the ectoparasitic mite Varroa destructor, and with issues related to poor queen quality (particularly premature queen replacement), which often result in decreased colony productivity and increased risk of mortality. We aimed to investigate how sublethal exposure to beekeeper-applied miticides affects honey bees at both the individual (queen) and colony levels. We did so by comparing the growth (comb built, brood produced, food stored, and worker population), queen supersedure rates, and winter survival probabilities of colonies that were headed by queens that were raised in either miticide-laden or miticide-free beeswax cups then housed in hives that were either treated with miticides or left untreated. Contrary to our prediction, we found that treated colonies headed by queens raised in miticide-laden beeswax built significantly more worker and drone comb, and stored more food, than any other colony treatment. We did not, however, observe any other significant effect of colony treatment on the amount of brood production, worker population size, queen supersedure rate, or colony winter survival. Thus, we failed to observe a direct negative effect of miticide exposure at the colony level. More studies are needed to further test the potentially detrimental synergistic effects of in-hive miticides on honey bee health at the colony level.

Keywords: Apis mellifera; Coumaphos; Honey bee queen; Tau-fluvalinate; Supersedure; Varroa destructor

Introduction

Pollinator health continues to be a topic of major interest worldwide, particularly due to the rapid population decline of both native and managed pollinator species [1-5]. The honey bee (Apis mellifera) is arguably the most important insect pollinator of major agricultural crops, contributing an estimated $200 billion to the global economy annually, $17 billion in the United States alone [6-8]. Despite their importance to agriculture, the number of managed honey bee colonies available for pollination has decreased steeply in the last decade, threatening the production of many bee-dependent crops nationwide [9,10]. This decline has been attributed to the many health issues facing honey bees today—problems caused by pathogens and parasites, the use of in-hive chemicals to treat for these ailments, the exposure of colonies to agricultural pesticides and genetically modified crops during foraging, and poor beekeeping practices [11-13]. Moreover, recent surveys from several commercial beekeeping operations across the United States reported that the most common causes of colony losses included poor queen quality, problems associated with the parasitic mite Varroa destructor, poor nutrition and starvation, and other less impactful factors [14,15]. The combined information from these reports suggests that the increased use of in-hive chemicals to combat Varroa mites has coincided with a general decrease in colony health that may lead to increased colony losses.

For the last two decades, Varroa mites have been controlled in the United States primarily with two in-hive miticides: the pyrethroid tau-fluvalinate (Apistan®) and the organophosphate coumaphos (Checkmite+). These chemicals are administered in colonies as miticide-impregnated strips placed between frames of brood, and kept in the hive for several weeks [16]. Despite their efficacy when first approved for use in apiaries, mites quickly developed resistance to both fluorvalinate [17-20] and coumaphos [21]. Continuous application of these lipophilic chemicals has had their permanent presence in the wax comb [22-25], especially in commercial beekeeping operations [26,27]. Even though therapeutic concentrations of fluorvalinate and coumaphos have been reported to have low toxicity to bees [28,29]-likely because of the bees’ rapid ability for detoxification by cytochrome P450 monooxygenases [30,31]-several studies have linked the use of these miticides with a decrease in honey bee colony health (see above). Likewise, the increased use of these miticides to control Varroa mites has coincided with an increase in problems relating to poor queen quality [22,32-35]. In fact, beekeepers continue to report issues with lower queen lifespan due to rapid supersedeure—when the workers in a honey bee colony replace their mother queen with a new sister queen—as well as the inability of colonies to naturally raise new queens in a timely fashion [36-38].

Numerous studies have explored the effects of either fluorvalinate or coumaphos on the reproductive quality of honey bees. For example, persistent application of fluorvalinate has been shown to decrease the sexual competitiveness and size of drones [39-41]. Likewise, colonies treated with high doses of fluorvalinate have resulted in disturbed oviposition and rapid queen losses [22], low queen weight [32], and poor queen rearing success [34]. Similarly, prolonged persistence of coumaphos in a colony, even at low doses, has been shown to cause low

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queen body weight \[32,33,35\], low queen ovary weight \[32\], low queen-rearing success \[33\], as well as high queen mortality \[32\]. This evidence demonstrates that fluvalinate and coumaphos use have potentially damaging effects to honey bee health, particularly individual drones and queens.

Honey bee queens are produced commercially by ‘grafting’ (the physical transfer of worker larvae from worker cells into plastic or beeswax cups). Once larvae are grafted, the cups are positioned vertically inside queenless colonies where a large population of young nurse workers feed royal jelly to the queen-destined larvae until they pupate and their wax cells get sealed \[42\]. One study of commercial queen rearing operations found higher accumulations of fluvalinate and coumaphos in queen cells compared to the surrounding beeswax, which suggests that queen larvae are differentially exposed to those chemicals during development \[32\]. The aforementioned studies have looked at the effects of either fluvalinate \[29,32\] or coumaphos \[32-35\] on honey bee reproductive health. But to date, no studies have looked at the combined effects of both miticides on the health of honey bee queens and the colonies they head, which is a critical consideration knowing that both compounds are ubiquitous within managed beehives in the US \[27\] and have been shown to exhibit negative synergetic effects on individual bees \[31,43\].

In this study, we attempted to address the potential issues associated with exposure to combinations of tau-fluvalinate and coumaphos by both, developing honey bee queens, and new colonies headed by these queens. We did so by establishing new colonies that were headed by queens reared in either miticide-laden or miticide-free beeswax cups, and then housed in hives that were either treated with those same miticides or left untreated. We subsequently measured several variables of colony growth, as well as queen supersEDURE events and winter survivorship, to determine if exposing colonies to miticides in the queen-rearing environment, the hive environment, or both, has an effect on overall queen and colony health. Doing so will identify a potentially important mechanism of colony losses and a means to mitigate them.

**Methods**

**Study site and bees**

This study was conducted at the Lake Wheeler Honey Bee Research Facility of North Carolina State University in Raleigh, North Carolina (35°43’ 27”, -78°40’33”). All the colonies from which we created packages of bees were headed by naturally mated ‘Italian’ queens (*Apis mellifera ligustica*). We used a single source colony to graft all experimental queens, and thus, all queens were sisters to each other.

**Beeswax queen cups**

We used standard queen-rearing plastic cups that we coated with molten beeswax obtained from Kenya (Burt’s Bees, Morrisville, NC), a country where honey bees are not treated with miticides for Varroa control \[44\]. Moreover, because of the extremely low tolerance for hive and other pesticides in their products, cosmetic companies maintain very strict standards for purchasing pesticide-free beeswax. We coated each plastic cup with \( \sim 240 \) mg of molten beeswax, covering the bottom, inner, and outer sides of the cup. To create miticide-free beeswax cups, we simply submerged each cup into the miticide-free molten wax. To create beeswax cups impregnated with miticides, we added \( \sim 20.4 \) mg of fluvalinate (PESTANAL analytical standard, Sigma-Aldrich, St. Louis, MO) and \( \sim 9.4 \) mg of coumaphos (PESTANAL analytical standard, Sigma-Aldrich, St. Louis, MO) to 100 g of miticide-free beeswax to obtain a total miticide concentration of \( 204 \) ppm of fluvalinate and \( 94 \) ppm of coumaphos in the wax. These total concentrations were chosen from the reported maximum concentrations of these two miticides found in wax comb samples obtained from over \( 250 \) beekeeping operations recently surveyed across the United States \[27\].

**Queen rearing**

On 16 May 2011, we reared experimental queens from one-day-old worker larvae from a single source colony following standard queen-rearing grafting methods \[45\]. Briefly, we transferred each larva into a beeswax-coated plastic cup that was either miticide-free or impregnated with fluvalinate and coumaphos in the concentrations mentioned above. We then placed the larvae grafted in the miticide-laden beeswax cups into one “swarm box” colony (i.e., a queenless colony with nurse workers to raise new queens), and the larvae grafted in the miticide-free beeswax cups into a separate swarm box colony. These queen rearing colonies, as well as all initial colonies in the study population, were not treated with either compound for mite control and thus had minimal (albeit probably non-zero) levels of both miticide. Once the mature queen cells were sealed, they were individually placed inside small “nucleus” colonies containing about \( 1,000 \) workers, where each virgin queen was allowed to emerge from her cell, mate naturally, and eventually commence oviposition. The mated queens were labeled with a paint mark on the thorax and were finally introduced into new experimental colonies (see below) on 9 June 2011. We raised a total of \( 40 \) naturally mated experimental queens, half of them reared in miticide-free beeswax cups and the other half reared in miticide-laden beeswax cups.

**Establishment of new experimental colonies**

On 9 June 2011, we created \( 40 \) packages of bees from larger, unrelated source colonies that had never been treated with fluvalinate or coumaphos for mite control. Each package of bees was queenless and contained a standard 2 lbs of bees, or about \( 7,000 \) individuals (1.0 kg of bees contains approximately \( 7,700 \) individuals) \[46\]. We then shook the bees from each package into a 10-frame hive box containing alternating frames with either partial or full wax foundation. Full-wax foundation only promotes worker comb construction, whereas partial-wax foundation enables the workers to construct either worker or drone foundation depending on the colony’s needs. A total of \( 40 \) ten-frame hives bodies were used, \( 20 \) of which were newly constructed and thus had never been exposed to any in-hive chemical contamination, whereas the other \( 20 \) hives had been used in apiaries for several years, and thus, might have had residual levels of chemical pesticides. Likewise, we used either newly-purchased frames or used, rewired frames, placing them into new or used hive bodies, respectively. We then introduced one of the mated experimental queens into each newly established colony. The \( 40 \) hives were established in the same apiary and placed in alternating order by treatment.

**Miticide treatment of experimental hives**

The \( 20 \) colonies established in new beekeeping equipment were left untreated throughout the experiment. The other \( 20 \) colonies housed in used beekeeping equipment were treated with miticides after colony establishment. To treat colonies, we placed half a strip of Apistan® (Bayer Corporation, Shawnee Mission, KS) and half a strip of Checkmite® (Bayer Corporation, Shawnee Mission, KS) between brood frames following the therapeutic dosages suggested by the manufacturers. We treated colonies twice, once on 22 June 2011 and then again on 8 July 2011, removing the strips two weeks after each
application. In summary, we created a total of 40 experimental hives, 10 each belonging to one of four experimental groups: colonies headed by queens raised in miticide-free beeswax cups and housed in new untreated hives (Treatment 1); colonies headed by queens reared in miticide-free beeswax cups and housed in hives treated with fluvinate and coumaphos strips (Treatment 2); colonies headed by queens that were raised in miticide-laden beeswax cups and housed in untreated hives (Treatment 3); and colonies headed by queens that were raised in miticide-laden beeswax cups and housed in treated hives (Treatment 4).

Measurements of colony growth

To test the effects of miticides in queen-rearing and hive environment on colony growth, we took monthly measurements from each experimental colony from 9 June 2011, the day of colony establishment (Day 0), through 14 October 2011, the last day of data collection (Day 127). We did so by using a gridded wooden frame containing 136 1-in² squares to measure several characteristics of colony growth, as described previously [47]. We began by estimating the total area of newly built worker and drone comb, the total area of sealed worker and drone brood, and the total area of stored food (including honey and pollen). We then estimated worker population size by uniformly sampling each frame in the hive, counting the number of bees in 20 evenly spaced 1-in² squares on both sides of the frame, extrapolating the resulting counts to estimate the total worker population covering the entire frames, and adding to those values an estimate of the number of workers found on the inner walls of the hive.

Measure of queen supersedure and winter survival

To test the effects of queen-rearing and hive environment on queen supersedure and winter survival rates, we recorded any episode of queen supersedure in newly established colonies. We defined a successful supersedure event as the presence of a laying, unmarked queen and the absence of the marked, experimental queen. Likewise, we monitored colony survival throughout the season and into the early spring the following year by listening for bees buzzing in the hive to determine which colonies were still alive on 1 March 2012 (Day 266 after colony establishment).

Statistical analysis

To test the effect of colony treatment on colony growth, we performed a generalized linear mixed model using the GLIMMIX procedure on the SAS statistical software (SAS Institute Inc., Cary, NC). Because the measured variables (e.g., amount of comb built, amount of brood produced, amount of food stored, and adult worker population size) were taken continually from the same colonies over time, we built the model to include the fixed effects of queen-rearing environment, hive environment, or both, on the growth patterns of newly established colonies. We defined a successuliferous event as the presence of a laying, unmarked queen and the absence of the marked, experimental queen. Likewise, we monitored colony survival throughout the season and into the early spring the following year by listening for bees buzzing in the hive to determine which colonies were still alive on 1 March 2012 (Day 266 after colony establishment).

Results

We grafted larvae into a total of 90 miticide-free beeswax cups, and 90 miticide-laden beeswax cups. Of these, nurse workers initiated the queen-rearing process in 74 miticide-free cups (82% queen-rearing initiation success), and 51 miticide-laden cups (56% initiation success). Of the queen cups that got initiated by workers, 34 queens raised in miticide-free beeswax cups, and 26 queens raised in miticide-laden beeswax cups, emerged from their cells and mated successfully. We should strongly caution, however, that these data are not directly comparable to previous estimates of grafting success, as the two treatment groups of queens were reared in separate colonies to avoid cross-contamination. Our purpose here was to obtain viable, laying queens rather than assess the process by which they were raised.

The effects over time of exposure of the miticides fluvinate and coumaphos in either the queen-rearing environment, the hive environment, or both, on the growth patterns of newly established honey bee colonies are shown in Figure 1. Colonies headed by queens raised in miticide-laden beeswax cups and housed in hives that were treated with miticides constructed on average twice as much worker comb (Figure 1a; \(F_{1,22}=4.48, P=0.04\)) and –10 times more drone comb (Figure 1b; \(F_{1,22}=8.72, P=0.009\)), and stored approximately 60 percent more honey and pollen (Figure 1c; \(F_{1,22}=4.147, P=0.05\)), compared to any other colony treatment. There was no significant effect of miticide exposure in the queen-rearing environment or hive environment on the production of worker brood (Figure 1d; \(F_{1,22}=0.13\), the production of drone brood (Figure 1e; \(F_{1,22}=1.30, P=0.27\)), or the population of adult workers present in the hive (Figure 1e; \(F_{1,22}=1.82, P=0.41\)). Thus overall, we found that for some parameters measured, but not all, colonies that were exposed to miticides in the queen-rearing and hive environment grew larger and stronger throughout the season than those headed by any other colony treatment.

We found no significant positive effect of colony treatment on the winter survival probability of newly established colonies. Of the 40 colonies that were established on 9 June 2011 (i.e., 10 colonies per treatment group), a total of only 5 colonies were alive by 1 March 2012. Of these, 2 colonies were headed by queens raised in miticide-free beeswax cups and housed in untreated hives, 2 colonies were headed by queens raised in miticide-laden beeswax cups and housed in untreated hives, and one colony was headed by queens raised in miticide-laden beeswax cups and housed in treated hives. None of the colonies headed by queens raised in miticide-free beeswax cups an housed in treated hives survived through the winter. By 14 October 2011 (Day 127), almost twice as many colonies (7 vs. 4) housed in untreated hives were still alive compared to colonies housed in treated hives (Figure 2). But overall, survivorship of colonies decreased steadily and similarly over time for all treatment groups, and colony treatment had no significant effect on the probability of colony winter survival (\(\chi^2=0.06; P=0.80\)).

Finally, we observed a total of seven supersedure events after colony establishment on 9 June 2011 (Table 1). However, there was no significant effect of colony treatment on the likelihood that the workers in a colony superseded the queen (2-tailed test, \(P=0.428\)). Interestingly, no supersedure events were recorded after 17 August 2011, indicating that colonies replaced their mother queen within just a few weeks after colony establishment.
Figure 1: Growth patterns for honey bee colonies established on 9 June 2011 (Day 0), each belonging to one of four treatments based on whether their colonies were headed by queens that were raised in either miticide-laden or miticide-free beeswax cups, and their hives were treated with miticides or left untreated. Upon establishment, each colony received a standard 2 lbs of bees, or approximately 6,950 workers. Colony growth was monitored through 14 October 2011 (Day 127). See “Methods” for details on colony set up. Data are presented as the mean ± S.E.M.

Table 1: Episodes of queen supersedure (i.e., when the workers in a honey bee colony replace their mother queen with a new sister queen) in colonies that belonged to one of four treatment groups based on whether their hives were treated with the miticides fluvalinate and coumaphos (following label therapeutic application protocols), and whether they were headed by queens that were reared in miticide-laden or miticide-free beeswax cups (see “Methods” for details). All colonies were established on 9 June 2011 and queen supersedure events were monitored through 14 October 2011.
sperm viability, and higher mating frequency, compared to queens raised in miticide-laden beeswax cups showed significantly lower sperm counts, lower pheromones between queens raised in miticide-free versus miticide-laden beeswax cups, and if so, to what degree workers inside the colony perceive these differences.

This study is the first to look at the combined long-term effects of both coumaphos and fluvalinate on the growth, queen supersedure rate, and wintering survival of honey bee colonies. Despite previous reports of detrimental effects of these miticides at the individual level, our results indicate that exposure of these miticides does not seem to cause detrimental problems at the colony level. In general, chemical treatment to control Varroa continues to be widely practiced by beekeepers, given that, if left untreated, persistent mite infestations generally lead colonies to succumb to "parasitic mite syndrome," which weakens them point of collapse, and even death [60]. Nevertheless, the use of fluvalinate and coumaphos appears to be on the decline due to the mite's wide-spread resistance to these chemicals [16] and thus alternative methods for Varroa control have been used successfully [61,62]. Therefore, a novel avenue of research regarding the effects of alternative mite-control methods on queen survival and colony growth should be pursued. Thus, future studies should focus not only on the sublethal effects of commonly-used miticides, but also the effects of alternative Varroa-control methods on colony productivity and longevity over several years, which are the true indicators of overall colony health.

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