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ORIGINAL RESEARCH ARTICLE

The effects of three acaricides on the developmental biology of small hive beetles (*Aethina tumida*).

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Summary

Small hive beetles (*Aethina tumida*, SHB) are an emerging problem for beekeepers internationally. Here we report how SHB development is affected by three acaricides, representing three chemical classes, that are used to control varroa mites (*Varroa destructor*): pyrethroids (fluvalinate - Apistan®), botanical extracts (thymol, camphor, menthol, eucalyptol - Apilife VAR®), and organophosphates (coumaphos - Checkmite+®). Our data indicate that the three acaricides vary in toxicity to SHB developmental stages. Apistan was toxic to feeding and wandering larvae but innocuous to adults while Apilife VAR only exhibited toxicity to perpetually-wandering larvae. Checkmite+ had the broadest toxicity, killing both larvae and adults. The three acaricides only affected pupal development with regard to d spent pupating. There was no increased mortality in pupae that were exposed to the acaricides as feeding or wandering larvae. Our data are useful for developing chemical controls for various developmental stages of SHB by demonstrating which developmental stages are most vulnerable to three chemical treatments.

Efectos de tres acaricidas sobre la biología de desarrollo del pequeño escarabajo de la colmena (*Aethina tumida*).

El pequeño escarabajo de la colmena (*Aethina tumida*, SHB) esun nuevo problema en la apicultura internacional. Este trabajo muestra cómo es afectado el desarrollo de SHB por tres acaricidas diferentes, que representan tres clases de productos químicos usados para el control del ácaro varroa (*Varroa destructor*): piretroides (fluvalinato - Apistan®), extractos de plantas (timol, alcanfor, mentol, eucaliptol - Apilife VAR®), y organofosforados (coumafos - Checkmite+®). Nuestros datos indican que los tres acaricidas varían en la toxicidad sobre las etapas de desarrollo de SHB. Apistan fue tóxico para la alimentación y el movimiento de las larvas pero inocuo para los adultos, mientras Apilife VAR sólo mostró toxicidad en el movimiento de las larvas. Checkmite + tubo la toxicidad más amplia, matando tanto a larvas como adultos. Los tres acaricidas sólo afectaron al desarrollo pupal en relación con la duración de la pupación. No hubo incremento de la mortalidad en pupas expuestas a los acaricidas durante la fase de alimentación o de movimiento de las larvas. Nuestros datos son útiles para el desarrollo de controles químicos en diferentes etapas de desarrollo de SHB al demostrar cuales etapas de desarrollo son las más vulnerables a los tres tratamientos químicos.

Keywords: acaricide, Apilife VAR, Apistan, Checkmite+, small hive beetle, *Aethina tumida*, development

Introduction

Small hive beetles (*Aethina tumida*, SHB) are an emerging problem for beekeepers internationally. Chemical control measures that target SHB adults or wandering larvae have been tested, but mostly with little success (Hood, 2004; Ellis 2005). To date, there have been no quantitative studies of chemical treatments on the various SHB developmental stages to

determine which stages are most vulnerable to treatment. We investigated how SHB development is affected by three acaricides, representing three chemical classes, that are used to control varroa mites (*Varroa destructor*): pyrethroids (fluvalinate - Apistan®), botanical extracts (thymol, camphor, menthol, eucalyptol - Apilife VAR®), and organophosphates (coumaphos - Checkmite+®). All are used in beehives as miticides. Checkmite+ is also used as an insecticide to control SHB. Our data are useful for developing future controls for SHB by demonstrating which developmental stages are most vulnerable to three chemical treatments.

Materials and Methods

All experiments were conducted from August 2004–January 2005 at the University of Georgia's honey bee research laboratory. All adult and larval SHB used in the studies were laboratory reared according to standard procedures (Mürrle and Neumann, 2004).

Acaricide effects on SHB oviposition and egg emergence were assessed by placing 6 unsexed adult SHB in a covered Petri dish with 2cm^3 of a mixture of pollen, honey, and Brood Builder™ (Dadant and Sons, Inc., Hamilton, IL, USA). Two microscope slides separated by a cover slip were placed in each Petri dish, and the Petri dishes were randomly divided into four treatment groups: 6cm^2 Apistan, 6cm^2 Checkmite+, 1 Apilife VAR tablet, or nothing (control). All Petri dishes had a 2cm diameter hole, covered by screen mesh, in their lids and were put collectively into covered plastic containers (~60 L volume) by treatment. One tablet of Apilife VAR was placed into the 60 L container for the Apilife VAR treatment. Therefore, adult SHB were exposed only to vapors from the Apilife VAR tablet through holes in the Petri dish lids. Apistan and Checkmite+ treatments were applied by placing 6cm^2 sections of strips in the Petri dishes. The untreated group was prepared in a similar manner but received no treatment. The containers were maintained at 25.6 ± 0.3°C, >80% humidity, and no light. The adults were allowed to oviposit for 48 h after which they were removed and the eggs oviposited between the slides counted. Four days later, the eggs were examined under a dissecting microscope to determine emergence. This procedure was replicated 14 times for oviposition behavior and 7 times for egg emergence.

Toxicity to feeding larvae was determined by placing a 24 h-old larva into plastic containers (6cm height x 4.9cm diameter, ~80 ml volume) with 1cm^3 food, and placing each unit to one of four groups: 1cm^2 Apistan, 1cm^2 Checkmite+, 1 tablet Apilife VAR, or untreated containers. This chemical assignment equaled the amount of chemical per SHB adult used in the oviposition study. Larval SHB were exposed only to Apilife VAR vapors by placing the uncovered 80 ml containers into a lidded 60 L container having one Apilife VAR tablet. Experimental conditions were maintained at 25.6 ± 0.3°C, ~38% humidity, and no light. All remaining experiments received the same treatment regimens and were maintained identically. The no. larvae reaching the wandering phase and time until reaching the wandering phase were recorded. All larvae reaching the wandering phase were placed individually into an 80 ml container of moist (>10% moisture by weight) soil to allow pupation. The no. SHB pupating and time until emersion from the soil as adults were recorded for each SHB. This procedure was replicated 50 times for each treatment.

Acaricide effects on pupation success of larvae treated during the wandering phase were determined by placing the wandering larva individually into an 80 ml plastic container with one of the four treatments. After 24h, the larvae were put individually into 80 ml soil containers to allow pupation. The no. SHB successfully pupating and the time until emersion from the soil as adults were recorded for each treatment. This procedure was replicated 50 times each treatment.

The longevity of wandering-phase larvae and of newly-emerged adults was determined by putting wandering larvae or adults individually into 80ml containers with one of the four treatments. SHB adults were given 1:1 honey: water *ad lib*. We recorded the no. d larvae and adults survived and this procedure was replicated 50 times for each larval and adult treatment.

For the proportion of eggs that did not hatch (*Table 1*), the data were first transformed using arcsin√proportion to stabilize the variance prior to analyses. For reporting in this paper we give the raw, untransformed means. Response variables for oviposition (*Table 1*), feeding larvae (*Table 2*), larvae 24 h post-feeding (*Table 3*), and longevity (*Table 4*) were analyzed by treatment using a randomized design ANOVA. Means were compared using Tukey's test and are presented in tables 1–4. All analyses were conducted using Statistica (2001).

Table 1. Effects of Apistan, Apilife VAR, and Checkmite+ on SHB oviposition behavior for 48 h. Data are mean ± s.e., n. Columnar data followed by different letters are different at $\alpha \leq 0.05$. Means were compared using Tukey's multiple range test.

Treatment	No. eggs on slides	Proportion of eggs that did not hatch
Control	70.4 ± 19.6, 14a	0.12 ± 0.05, 7a
Apistan	58.7 ± 17.4, 14a	0.05 ± 0.02, 7a
Apilife VAR	29.5 ± 10.3, 14a	0.14 ± 0.08, 7a
Checkmite+	19 ± 5.7, 14a	0.06 ± 0.02, 7a

Table 2. Effects of Apistan, Apilife VAR, and Checkmite+ on feeding SHB larvae. Data are mean ± s.e., n. Columnar data followed by different letters are different at a≤0.05. Means were compared using Tukey's multiple range test. na = not applicable.

Treatment	d. spent feeding	no. that reached wandering phase	d. spent pupating	no. that pupated
Control	11.3 ± 0.1, 43a	8.6 ± 0.2, 5a	22.7 ± 0.1, 43a	8.6 ± 0.2, 5a
Apistan	13.1 ± 0.4, 8b	1.6 ± 0.5, 5b	18.7 ± 0.2, 7b	1.2 ± 0.4, 5b
Apilife VAR	11.2 ± 0.1, 46a	9.2 ± 0.4, 5a	22.4 ± 0.1, 44a	8.8 ± 0.4, 5a
Checkmite+	na	0, 5c	na	0, 5c

Table 3. Effects of Apistan, Apilife VAR, and Checkmite+ on SHB larvae exposed to the acaracides 24 h post-feeding, during the wandering phase. Data are mean ± s.e., n. Columnar data followed by different letters are different at a≤0.05. Means were compared using Tukey's multiple range test.

Treatment	d. spent pupating	no. that pupated
Control	20.3 ± 0.1, 50a	10, 5a
Apistan	25.8 ± 0.2, 48b	9.6 ± 0.4, 5a
Apilife VAR	20.7 ± 0.1, 49a	9.8 ± 0.2, 5a
Checkmite+	22.1 ± 0.2, 45c	9 ± 0.3, 5a

Table 4. Effects of Apistan, Apilife VAR, and Checkmite+ on the longevity of SHB wandering larvae and adults. Data are mean ± s.e., n. Columnar data followed by different letters are different at a≤0.05. Means were compared using Tukey's multiple range test.

Treatment	d. larvae survived	d. adults survived
Control	29.3 ± 1.4, 50a	18.2 ± 0.8, 50a
Apistan	15.8 ± 0.6, 50b	19.9 ± 0.9, 49b
Apilife VAR	8.3 ± 0.7, 50c	16.5 ± 0.3, 49a
Checkmite+	5.1 ± 0.3, 50d	1, 50c

Results

For the overall model, the no. eggs oviposited on slides was significantly different ($F = 2.8$; df = 3, 52; $P = 0.05$). There was no treatment effect for the proportion of eggs oviposited on slides that failed to hatch ($F = 0.7$; df = 3, 24; $P = 0.58$) (*Table 1*).

Treatment significantly affected the d larvae spent feeding ($F = 35.1$; df = 2, 94; $P < 0.00$) and no. larvae reaching the wandering phase ($F = 194.4$; df = 3, 16; $P < 0.00$) (*Table 2*). Larvae feeding in the presence of Apilife VAR and no chemical fed fewer d than larvae exposed to Apistan. All larvae exposed to Checkmite+ died while feeding. More larvae feeding in the presence of Apilife VAR and no chemical survived to the wandering phase than larvae exposed to Apistan. There were significant treatment effects on d SHB spent pupating ($F = 75.2$; df = 2, 91; $P = 0.00$) and no. adults that emerged ($F = 260.2$; df = 3, 16; $P < 0.00$) when exposed to the treatments as feeding larvae (*Table 2*). Larvae feeding in the presence of Apilife VAR and no chemical pupated longer than larvae feeding in the presence of Apistan. Further, significantly more larvae feeding in the presence of Apilife VAR and no chemical survived to adulthood than larvae exposed to Apistan.

Treatment significantly affected d spent pupating when wandering larvae were exposed to the acaricides for 24 h post-feeding ($F = 272.7$; df = 3, 188; $P = 0.00$)(*Table 3*). Untreated wandering larvae and those exposed to Apilife VAR spent less time pupating than those exposed to Apistan or Checkmite+. Treatment did not affect no. SHB emerging from soil when exposed to the acaricides as larvae for 24 h post-feeding ($F = 2.5$; df = 3, 16; $P = 0.10$).

Larval ($F = 168.9$; df = 3, 196; $P = 0.00$) and adult ($F = 212.2$; df = 3, 194; $P = 0.00$) longevity were significantly affected by treatment (*Table 4*). Larvae and adults in the four treatment groups experienced significantly different longevities from one another (decreasing respectively in larvae – untreated, Apistan, Apilife VAR, Checkmite+ and in adults Apistan, untreated, Apilife VAR, and Checkmite+).

Discussion

Oviposition behavior was not significantly affected by the adult SHB treatment history although SHB adults exposed to Checkmite+ oviposited numerically fewer eggs than adults in the other three groups. This likely is because adult SHB exposed to Checkmite+ did not survive as long as those exposed to the other treatments. The absence of an effect of Apistan and Checkmite+ on egg hatch rate is not surprising, because the eggs never contacted the chemicals directly (they were oviposited between slides). Either the Apilife VAR vapors were non-toxic or eggs were protected from the vapors by being oviposited in the narrow space between two slides separated by a cover slip.

Overall, the acaricides we evaluated were most effective on feeding larva. This largely is due to the high toxicity of Apistan and Checkmite+ against feeding larvae. Apistan had a number of sub-lethal effects on feeding SHB larvae including an increase in the no. d spent feeding and decrease in the no. larvae reaching the wandering phase and developing into adults. In sharp contrast, we found post-feeding larvae in the wandering phase to be the SHB developmental stage most resistant to chemical treatment. Even when exposed to Checkmite+ for 24 h, wandering larvae successfully burrowed into the soil and emerged as adults in numbers comparable to those in the other treatment groups. Likewise, Apistan did not harm wandering larvae while it elicited a number of measurable effects in feeding larvae.

Despite that wandering SHB larvae were least affected by the three acaracides, they did exhibit increased mortality when exposed to the acaricides. All three acaricides accelerated larval mortality over that of larvae not exposed to any acaricide. In fact, the longevity of the longest-lived wandering larvae was only half that of larvae not exposed to an acaracide. Regardless, it is doubtful that SHB larvae reaching the wandering phase *in vivo* would be exposed to an acaracide long enough to cause any significant effect on their development.

In summary, our data indicate that the three acaricides vary in toxicity to SHB developmental stages. Apistan was toxic to feeding and wandering larvae but innocuous to adults. Apilife VAR only exhibited toxicity to perpetually-wandering larvae. Checkmite+ had the broadest toxicity, killing both larvae and adults. The acaracides only affected pupal development with regard to d spent pupating. There was no increased mortality in pupae that were exposed to the acaracides as feeding or wandering larvae. To conclude, our data are useful for developing chemical controls for various developmental stages of SHB by demonstrating which developmental stages are most vulnerable to chemical treatment.

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