Susceptibility of the small hive beetle, Aethina tumida (Coleoptera: Nitidulidae), to insecticides and insect growth regulators

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Original article

Susceptibility of the small hive beetle, *Aethina tumida* (Coleoptera: Nitidulidae), to insecticides and insect growth regulators

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Abstract – The small hive beetle, *Aethina tumida* Murray, has become an important pest of the honeybee, *Apis mellifera* L., in the USA. In this study, we assessed the susceptibility of this pest to 14 selected insecticides and four insect growth regulators (IGRs). The results indicated that the small hive beetle (SHB) was selectively susceptible to several classes of insecticides. The lethal concentration for 50% mortality (LC_{50}) to adult SHBs was 0.53, 0.53, and 0.54 μg/vial for fenitrothion, chlorpyrifos, and methomyl, respectively. However, against the larval stage, fenitrothion was the most toxic with an LC_{50} of 0.89 μg/vial. Chlorpyrifos had an LC_{50} of 1.64 μg/vial which was similar to the LC_{50} of 1.21 μg/vial for fluvalinate and 2.24 μg/vial for methomyl. Overall, these insecticides were found to be more toxic to SHBs than the organophosphate coumaphos which is currently used for control of SHB populations. Among the IGRs tested, fenoxycarb and methoprene were the most effective on early instar larvae with an LC_{50} of 30.20 and 61.89 μg/vial, respectively. None of the IGRs were found to adversely affect the development of third–fourth instar larvae of the SHB. The susceptibility of the SHB was also assessed in soil bioassays, and the patterns of responses were similar to those reported with the glass-vial bioassays. Our data provided useful insights and baseline in the development of an effective pest management strategy for the SHB in honeybee colonies. However, these pesticides should be used in a way that minimizes honeybee exposure and meets safety requirements for human consumption of honey products.

small hive beetle / honeybee / insecticides / insect growth regulators

1. INTRODUCTION

The honey bee, *Apis mellifera* L., is of great economic importance not only for honey production but also for crop pollination (Robinson et al. 1989). The added value of crops in the USA pollinated by honeybee was estimated at $14.6 billion annually (Morse and Calderone 2000; Klein et al. 2007). Honeybee populations have significantly decreased over the past years due to various arthropod pests and pathogens (Ambrose et al. 2000; Ellis et al. 2010; Di Prisco et al. 2011). The most serious pests include the Varroa mite (*Varroa destructor* Anderson and Trueman), the tracheal mite (*Acarapis woodi* Rennie), and the small hive beetle (SHB) (*Aethina tumida* Murray). The SHB is native to sub-Saharan Africa, and entered the USA through Florida in 1998. It has since spread into more than 30 other states

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(Elzen et al. 1999a). The environmental requirements needed for a complete life cycle and survival of the SHB are easily met within the wide geographical distribution of the European honeybee, *A. mellifera* (Brown et al. 2002). The rapid spread and high reproductive potential of this pest, both within honeybee colonies and in stored products, coupled with the ability to overwinter in honeybee clusters make it a serious threat to apiculture (Elzen and Neumann 2004). Damage to the honeybee colonies is caused mainly by the feeding of larvae of the SHB on honey, pollen, and live broods. They also tunnel and pierce wax combs and defecate in stored honey causing it to ferment, weep, and froth away from the cells (Sanford 1999). As a result, the SHB represents a serious threat to honeybee colonies in Southern USA (Ellis and Hepburn 2006). The SHB was also reported to be a potential vector of honeybee viruses (Eyer et al. 2009).

The current chemical control methods for the SHB include the use of plastic strips of the organophosphorus insecticide, coumaphos (CheckMite®), under pieces of cardboard against adult SHB populations (Ellis and Delaplane 2007). Chemical treatments with coumaphos might leave residues in the honey products, were harmful to the bees, and failed to provide extended control of the pest (Elzen et al. 1999b; Hood 2000). In addition, a soil drench under infested colonies with permethrin (Gard-Star 40% EC) has not been effective because it controls only few beetle larvae unless application is correctly timed (Schmolke 1974; Hood 2000). Several trapping devices were also developed for control of the SHB. These include the Hood beetle trap, the Freeman beetle trap, and the West beetle trap among others. All these traps typically use an attractant (often apple cider vinegar) and a killing agent (mineral oil); they are not stand-alone control measures of the SHB but provide some level of reduction of the SHB populations (Hood 2004). In this paper, we widen the search for compounds which might be effective against the larvae (during wandering phase prior pupation) and adults and thus might be useful in the development of an integrated pest management strategy for the SHB.

2. MATERIALS AND METHODS

2.1. Insect collection

Multiple field collections of adult SHBs from infested hives were made from the apiary at FAMU Research and Cooperative Extension Center, Quincy, FL and from Rish Tupelo Apiary, Wewahitchka, FL. They were brought to the laboratory, watered, and fed with a honey-fortified artificial diet and pollen substitutes (Global Patties, Butte, MT).

2.2. Chemicals

All insecticides and insect growth regulators were technical-grade samples (>90% purity) used as supplied by the manufacturers. The insecticides included the organophosphates fenitrothion, chlorpyrifos, malathion, parathion, phosmet, diazinon, metamidophos, and coumaphos (Chemical Service, West Chester, PA), the carbamates baygon, oxamyl, and methomyl (Chemical Service, West Chester, PA), the pyrethroids fluvalinate and cypermethrin (FMC, Princeton, NJ), and the organochlorine endosulphan (FMC, Philadelphia, PA). The insect growth regulators screened included tebufenozide, methoprene, cyromazin, and fenoxycarb (Sigma-Aldrich, Saint Louis, MO).

2.3. Glass-vial bioassays

The procedure used in this bioassay was described by Kanga and Plapp (1995). In this procedure, 20-mL glass scintillation vials were treated with 0.5 mL solution of each of the test insecticides or IGRs in acetone. Concentrations of 1.0×10^4, 1.0×10^5, 1.0×10^6, 1.0×10^7, 1.0×0^8 μg per vial for insecticides and 1.0×10^2, 1.0×10^3, 1.0×10^4, 1.0×10^5, 1.0×10^6 μg per vial for IGRs were tested. All insecticides or IGRs were diluted in acetone to get the desired concentrations for bioassays. The vials were rolled until the acetone evaporated and the insecticides were coated on the inner surfaces. Vials treated with acetone only were used as controls. Three last instar

larvae or two adults were treated at each dose of the insecticides at room temperature (25°C), and mortality was determined 24 h after exposure. The treatments were replicated ten times, and about 210 adults or larvae of the SHB were tested per insecticide and IGR. Adults or larvae that were unable to walk a short distance (up to 10 mm) when released were considered dead.

2.4. Soil bioassay

Samples of oven-sterilized (160°C, 1 h) soil (20 g) were placed inside plastic cups (3.0×1.5 cm). The soil was humidified by sprinkling 2 mL of distilled water and allowed to settle for 30 min under a fume hood. A dilution ratio (*v*/*v*) from 1:1 to $1:10^6$ was tested for each insecticide and IGR in acetone. All chemicals were diluted in acetone to get the desired concentrations for bioassays, and the same amount of acetone without chemicals was used for controls. One milliliter of each concentration of the chemical was pipetted into the cups. Soil samples treated with acetone only were used as controls. Four last instar larvae of the SHB were placed in the cups, covered with a perforated lid, and held in a Percival Scientific Incubator (27±1°C, 85% RH, and 13:11-h L/D photoperiod) in the laboratory. There were four replicates per treatment, and the experiments were repeated on three different dates. About 192 insects were tested per experiment. Mortality of larvae of the SHB was recorded at 4, 7, and 14 days posttreatments.

2.5. Statistical analyses

The concentration–mortality data were subjected to Probit analysis to obtain the lethal concentrations (Russell et al. 1977). The control mortality was never greater than 5%, and data were corrected using Abbott's (1925) formula. Differences among insecticides were considered not significant if the 95% confidence limit of their toxicity ratio at the LC_{50} bracketed 1.0 (Robertson and Preisler 1992). Percentage mortality was also adjusted for control mortality (Abbott 1925), and data from each time group were subjected to Probit analysis (logistic transformation) to generate lethal time response (LT) (SAS Institute 1996). Toxicity values (LC_{50}s) of the insecticides were compared to those of coumaphos (currently recommended to beekeepers for control of the SHB).

3. RESULTS

3.1. Effect of insecticides in glass-vial bioassays

Toxicity data indicated that adults and larvae of the SHB were susceptible to all insecticides tested, and the toxicity varied significantly between developmental stages of the SHB (Table I). The organophosphorus insecticides fenitrothion and chlorpyrifos were the most toxic, while methamidophos was the least efficacious in killing the SHB. Chlorpyrifos, fenitrothion, and parathion were significantly more toxic [(based on the failure of the 95% confidence limit of their toxicity ratio at the LC_{50} to bracket 1.0 (Robertson and Preisler 1992)] to adult SHBs than coumaphos, the insecticide currently being used to control the SHB populations.

Fenitrothion and malathion had similar toxicity as coumaphos to SHB (Table I). Among the carbamate insecticides tested, only methomyl was more toxic to adult SHBs, and the response was statistically different from that of coumaphos (Table II). All the other carbamate insecticides tested were less toxic to larval SHBs than coumaphos.

The pyrethroids fluvalinate and cypermethrin were equally toxic to both developmental stages of the SBH as coumaphos (Table III). The cyclodiene, endosulfan, was less toxic to the SHB than coumaphos (Table III).

3.2. Effect of insect growth regulator in glass-vial bioassays

First instar larvae of the SHB were generally more susceptible to IGRs than later instar larvae (Table IV). They were 2.6-, 1.5-, 1.4-, and 4.3-fold more susceptible at the LC_{50} level for fenoxycarb, cyromazin, tebufenozide, and methoprene, respectively. Larvae of the SHB appeared to be more susceptible to fenoxycarb than to methoprene.

Table I. Responses of adults and larvae of *Aethina tumida* to organophosphorus insecticides in the laboratory.

Insecticides	Life stage	N^a	Slope±SE	$LC_{50}{}^b$ (95% CL)	Toxicity ratio[c] (95% CL)
Chlorpyrifos	Adults	264	3.58±0.47	0.53 (0.29–0.88)	3.03 (1.65–5.12)
	Larvae	264	1.57±0.16	1.64 (0.94–2.24)	0.80 (0.62–1.76)
Fenitrothion	Adults	320	3.86±0.5	0.53 (0.45–0.62)	3.03 (1.85–5.52)
	Larvae	384	1.79±0.15	0.89 (0.71–1.10)	1.48 (0.93–2.31)
Parathion	Adults	264	2.46±0.28	0.68 (0.54–0.84)	2.37 (1.32–4.83)
	Larvae	231	2.75±0.31	1.46 (1.19–1.80)	0.90 (0.73–1.85)
Malathion	Adults	280	2.59±0.20	0.84 (0.62–1.14)	1.92 (0.99–2.87)
	Larvae	424	1.75±0.14	1.05 (0.63–1.71)	1.26 (0.68–2.05)
Phosmet	Adults	264	2.48±0.20	0.88 (0.67–1.17)	1.83 (0.97–3.20)
	Larvae	264	1.46±0.10	0.89 (0.71–1.10)	1.48 (0.91–2.23)
Coumaphos	Adults	304	1.98±0.19	1.61 (0.98–2.58)	–
	Larvae	264	1.73±0.18	1.32 (0.92–1.89)	–
Diazinon	Adults	231	2.68±0.30	1.22 (0.98–1.50)	1.31 (0.81–2.11)
	Larvae	320	1.33±0.13	1.49 (0.92–2.44)	0.88 (0.69–1.82)
Methamidophos	Adults	240	2.73±0.32	1.62 (1.30–2.02)	0.99 (0.83–1.93)
	Larvae	297	2.17±0.23	5.69 (4.51–7.22)	0.23 (0.12–1.34)

[a] Number of larvae and adults of small hive beetle tested

[b] Concentrations are expressed in micrograms per vial of the insecticides tested

[c] Toxicity ratios were calculated by dividing the LC_{50} for coumaphos by that of the other insecticides

Tebufenozide and cyromazin were equally toxic to first and fourth instar larvae of the SHB (Table IV).

3.3. Effect of insecticides in soil bioassays

Toxicity data indicated that SHB populations were susceptible to the insecticides tested using soil bioassays, and the mortality increased significantly over time (Table V). The toxicity of chlorpyrifos was significantly higher than that of fenitrothion. Further, chlorpyrifos was 2.48-fold and 6.97-fold more toxic than fenitrothion at 4 and 7 days posttreatment, respectively (Table V). The patterns of susceptibility of the SHB to chlorpyrifos and fenitrothion were similar to our previous results with the glass-vial bioassays.

Table II. Responses of adults and larvae of *Aethina tumida* to carbamate insecticides in the laboratory.

Insecticides	Life stage	N^a	Slope±SE	$LC_{50}{}^b$ (95% CL)	Toxicity ratio[c] (95% CL)
Methomyl	Adults	264	2.81±0.34	0.54 (0.40–0.73)	2.98 (1.42–3.83)
	Larvae	297	1.97±0.20	2.24 (1.76–2.87)	0.59 (0.41–1.52)
Propoxur	Adults	280	2.16±0.22	1.74 (1.24–2.41)	0.92 (0.78–1.87)
	Larvae	336	1.84±0.17	3.17 (2.31–4.45)	0.41 (0.21–1.47)
Oxamyl	Adults	240	2.61±0.30	1.96 (1.31–2.91)	0.84 (0.63–1.73)
	Larvae	264	1.46±0.15	1.46 (0.94–2.24)	0.90 (0.70–1.81)

[a] Number of larvae and adults of small hive beetle tested

[b] Concentrations are expressed in micrograms per vial of the insecticides tested

[c] Toxicity ratios were calculated by dividing the LC_{50} for coumaphos by that of the other insecticides

Table III. Responses of adults and larvae of *Aethina tumida* to pyrethroid and organoclorine insecticides in the laboratory.

Insecticides	Life stage	N^a	Slope±SE	$LC_{50}{}^b$ (95% CL)	Toxicity ratio[c] (95% CL)
Pyrethroids					
Cypermethrin	Adults	320	1.85±0.17	1.96 (1.56–2.47)	0.82 (0.61–1.75)
	Larvae	360	1.35±0.12	1.91 (0.98–4.04)	0.69 (0.52–1.68)
Fluvalinate	Adults	280	2.29±0.34	2.20 (1.49–3.25)	0.73 (0.59–1.69)
	Larvae	264	2.24±0.24	1.21 (0.96–1.52)	1.09 (0.65–1.98)
Cyclodiene					
Endosulfan	Adults	360	2.57±0.27	8.70 (7.17–10.61)	0.19 (0.10–1.29)
	Larvae	297	1.91±0.20	6.43 (5.01–8.33)	0.21 (0.13–1.32)

[a] Number of larvae and adults of small hive beetle tested

[b] Concentrations are expressed in micrograms per vial of the insecticides tested

[c] Toxicity ratios were calculated by dividing the LC_{50} for coumaphos by that of the other insecticides

3.4. Effect of insect growth regulators in soil bioassays

Data in Table VI indicated that fenoxycarb was more toxic to the larvae of the SHB and significantly different from cyromazin after days 4, 7, and 14. However, the levels of toxicity were lower until day 14, which may indicate the slow acting nature of these compounds. In addition, the lethal time for 90% mortality (LT_{90}) was 11.46 days for fenoxycarb and 15.37 days for cyromazin. Thus, it took more than 10 days for these compounds to kill 90% of the small hive beetle populations.

4. DISCUSSION

Results indicated that the SHB was selectively susceptible to several classes of insecticides and suggested potential new avenues for control. Among the eight organophosphorus insecticides tested, fenitrothion, chlorpyrifos, and parathion were more effective in controlling the SHB than was coumaphos, which is

Table IV. Responses of first and fourth instar larvae of *Aethina tumida* to insect growth regulators in the laboratory.

Insecticides	Life stage	N^a	Slope±SE	$LC_{50}{}^b$ (95% CL)	Toxicity ratio[c] (95% CL)
Fenoxycarb	1st instar	105	2.24±0.39	30.20 (22.66–39.56)	2.55 (1.38–4.98)
	4th instar	160	2.09±0.27	77.05 (55.81–108.11)	–
Cyromazin	1st instar	105	2.11±0.35	30.72 (18.46–47.70)	1.54 (0.95–2.28)
	4th instar	160	1.50±0.19	47.29 (24.45–64.79)	–
Tebufenozide	1st instar	105	2.96±0.54	25.94 (19.19–34.29)	1.45 (0.87–2.12)
	4th instar	160	1.55±0.20	37.52 (24.84–46.90)	–
Methoprene	1st instar	105	2.31±0.38	61.89 (39.53–94.69)	4.29 (2.84–6.98)
	4th instar	160	1.89±0.28	266.05 (191.37–383.63)	–

[a] Number of larvae and adults of small hive beetle tested

[b] Concentrations are expressed in micrograms per vial of the insect growth regulators tested

[c] Toxicity ratios were calculated by dividing the LC_{50} for 4th instar by that of 1st instar

Table V. Responses of last instar larvae of *Aethina tumida* to chlorpyrifos and fenitrothion in soil bioassays.

Insecticides	Life stage	N^a	Slope±SE	$LC_{50}{}^b$ (95% CL)	Toxicity ratio[c] (95% CL)
After 4 days					
Chlorpyrifos	Larvae	300	5.01±0.69	3.71 (2.97–4.59)	2.48 (1.32–4.92)
Fenitrothion	Larvae	300	6.62±1.00	9.20 (7.61–11.18)	–
After 7 days					
Chlorpyrifos	Larvae	300	6.36±1.23	0.73 (0.57–0.89)	6.97 (3.42–13.63)
Fenitrothion	Larvae	300	3.94±0.48	5.09 (4.00–6.50)	–

[a] Number of larvae and adults of small hive beetle tested

[b] Concentrations are expressed in micrograms per cup of the insecticides tested

[c] Toxicity ratios calculated by dividing the LC_{50} for fenitrothion by that of chlorpyrifos

currently used for in-hive treatments. The carbamate methomyl and the pyrethroids, cypermethrin and fluvalinate, might also be used to control the SHB populations. In similar studies using feeding bioassays, Ellis and Delaplane (2007) reported that fluvalinate was toxic to feeding and wandering larvae but innocuous to adults, while coumaphos had the broader toxicity, killing both larvae and adults. Thus, chlorpyrifos, fenitrothion, parathion, fluvalinate, cypermethrin, and methomyl might also be used judiciously as bait stations within the beehives for control of the SHB. In soil treatments, chlorpyrifos was more effective against late instar larvae than permethrin, which is currently used in soil drench applications. The last instar larvae appeared to be the most vulnerable stage as the larvae seek suitable soil sites for pupation and therefore more accessible to treatments. Because of the deleterious effects of pesticides, any compounds to be used in or around the beehive for control of the SHB are required to be less toxic to honeybee populations and meet safety requirements for human consumption of honey products. However, Hardstone and Scott (2010) indicated that in general, honeybees were no more sensitive than other insect species to the six classes of insecticides (carbamates,

Table VI. Responses of last instar larvae of *Aethina tumida* to fenoxycarb and cyromazin in soil bioassays.

Insecticides	Life stage	N^a	Slope±SE	$LC_{50}{}^b$ (95% CL)	Toxicity ratio[c] (95% CL)
After 4 days					
Fenoxycarb	Larvae	312	2.19±0.28	356 (241–497)	5.36 (3.05–11.02)
Cyromazin	Larvae	312	3.83±0.56	1,910 (1,532–2,382)	–
After 7 days					
Fenoxycarb	Larvae	312	2.13±0.32	341 (204–500)	5.30 (2.98–10.85)
Cyromazin	Larvae	312	4.67±0.64	1,810 (1,277–2,497)	–
After 14 days					
Fenoxycarb	Larvae	312	1.64±0.41	40 (4–102)	7.93 (5.41–14.35)
Cyromazin	larvae	312	3.07±0.37	317 (230–422)	–

[a] Number of larvae of small hive beetle tested

[b] Concentrations are expressed in micrograms per cup of the insect growth regulators tested

[c] Toxicity ratios calculated by dividing the LC_{50} for cyromazin by that of fenoxycarb

nicotinoids, organochlorines, organophosphates, pyrethroids, and miscellaneous chemicals) they examined. The fact that fenitrothion, parathion, chlorpyrifos, and methomyl were more toxic than coumaphos to the SHB may suggest that lower concentrations of these chemicals could be used to achieve successful control measures, therefore reducing chemical usage and contamination of honey, honey products, and the environment.

The SHB is not restricted to honeybees and can reinvade hives from other sources; thus, a long-term management strategy is needed. Our data on insect growth regulators indicated that fenoxycarb and methoprene were more effective against early instar larvae than older larvae. Further, the toxicity of tebufenozide and cyromazin was similar between first and fourth instar larvae of the SHB. These chemicals could be used judiciously (sequential or rotational use) with other control measures to design an effective integrated pest management strategy. Because of the broad spectrum activity of the OP, carbamate, and pyrethroid insecticides and their adverse impacts on honeybees, there is merit of widening the search for alternatives to coumaphos. The search should include neonicotinoids, pyrazoles, pyroles, avermectins, and newer generations of insecticides. Hardstone and Scott (2010) indicated that while honeybees can be sensitive to individual insecticides, they were not a highly sensitive species to insecticides overall, or even to specific classes of insecticides. However, all pesticides should be used in a way that minimizes honeybee exposure.

Overall, this study provided useful insights and baseline data in the development of a cost-effective pest management strategy for the small hive beetle in the honeybee industry. However, studies of the toxicity of these insecticides to honeybees are yet to be conducted.

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Sensibilité du petit coléoptère des ruches, *Aethina tumida* (Coleoptera: Nitidulidae) envers les insecticides et les régulateurs de croissance d'insectes.

Abeille / ravageur / lutte / insecticide / régulateur de croissance / ennemi de la ruche

Anfälligkeit des kleinen Beutenkäfers *Aethina tumida* (Coleoptera: Nitidulidae) gegen Insektizide und Wachstumsregulatoren.

kleiner Beutenkäfer / Honigbiene / Insektizide / Wachstumsregulatoren

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