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Mechanisms of insecticide resistance in field populations of the varroa mite (Acari: Mesostigmata: Varroidae) in Florida

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The honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), is critical not only for honey production but also for crop pollination. More than 130 agricultural plants in the United States are pollinated by honey bees (McGregor 1976). The ectoparasitic mite *Varroa destructor* Anderson & Trueman (Acari: Mesostigmata: Varroidae) is the most serious threat to beekeeping worldwide (Cox-Foster et al. 2007; Calderón et al. 2009). This parasitic mite causes weight loss, malformation of wings, and a shortened life span in honey bees; the mite also serves as a vector of disease-causing agents. Without adequate control of *Varroa* infestations, bee mortality approaches 100% and colonies can perish within a few weeks (De Jong et al. 1982; Ball 1994; Kanga et al. 2010).

Miticides, especially fluvalinate (Apistan®; Bayer Corp., Kansas City, Missouri) and coumaphos (CheckMite®; Bayer HealthCare LLC, Shawnee Mission, Kansas), are the most cost-effective and widely used method of mite control for managed honey bee colonies. Resistance to these miticides has been documented (Elzen et al. 1998; Elzen & Westervelt 2002; Kanga et al. 2010). Identification of actual or potential resistance mechanisms is needed to maximize the likelihood of success in resistance management programs. Major mechanisms of resistance usually include enhanced metabolic degradation or decreased sensitivity of cholinergic or non-cholinergic neural target sites (Wang et al. 2002; Brogdon & McAllister 1999).

Female mites were collected from infested frames of sealed brood taken from honey bee colonies maintained in Wewahitchka, Florida. Drone and worker brood cells were opened, and female mites were collected from larvae and pupae by using a camel-hair brush. The mites were placed into glass scintillation vials (20 mL) containing honey bee larvae as a food source before miticide bioassays.

The mechanisms of resistance were determined by using known synergists as diagnostic probes (Kanga et al. 1996). The bioassay procedure was the modified glass vial technique of Kanga & Plapp (1995). The synergists used in these experiments were piperonyl butoxide (PBO), a mixed-function microsomal oxidase inhibitor; *S,S,S*-tributyl phosphorotrithionate (DEF), a putative inhibitor of esterases; triphenyl phosphate (TPP), an inhibitor of carboxylesterase; diethyl maleate (DEM), a glutathione S-transferase inhibitor (Grant et al. 1989); and formamidine as a target site synergist. The concentration of synergists in the bioassays was 50 µg per vial for PBO, 25 µg per vial for DEF, 50 µg per vial for DEM, 25 µg per vial for TPP, and 50 µg per vial for formamidine, which were the highest concentrations that were not toxic to the mite during pre-experimental runs (data not shown). Two sets of bioassays were conducted on each occasion. In set 1, mites were treated with a miticide and synergist mixture, and in set 2, mites were treated with the miticide alone. Vials treated with ethanol served as controls. Each miticide was tested using 8 concentrations (plus an ethanol control) with 5 replicates of 3 mites per vial. The experiments were repeated on 5 dates. All treated vials were held at room temperature (27 ± 1 °C) and 65% RH, and mite mortality was recorded 18 h after exposure. Mites that were unable to walk for a short distance (>5 mm) after gentle probing with a fine brush were considered dead.

Concentration–mortality data with and without synergists were subjected to Probit analysis using the POLO program (Russell et al. 1977). Percentage of mortality in the treatments was corrected for control mortality using Abbott's formula (Abbott 1925). The effects of synergists were calculated by dividing the LC50 for the miticide alone by the LC50 for the selected miticide and synergist mixture. The responses to synergists were considered not significant if the 95% confidence limit (CL) of the synergism ratio at the LC50 bracketed 1.0 (Robertson & Preisler 1992). A likelihood ratio test of equality was conducted to determine whether the regression lines of the 2 treatments were equal (i.e., whether the slopes and intercepts of the 2 lines were the same). A similar ratio test of parallelism was run to determine whether the regression lines were parallel (i.e., whether the slopes of the 2 lines were the same) (Robertson & Preisler 1992).

The levels of toxicity (LC50) to varroa mites of the miticide coumaphos alone and the coumaphos and DEF mixture were not significantly different (Table 1). In addition, dose–mortality regression lines for both treatments were equal (χ^2 = 1.68; df = 2; P = 0.43) and parallel (χ^2 = 0.52; df = 1; P = 0.47). The synergism ratio (SR) was 0.12-fold and therefore less than 1.0. These results suggest that enhanced metabolism by esterase was not a major factor of resistance in varroa mite populations of northern Florida.

The synergism ratio (SR = 0.35-fold) of coumaphos compared with coumaphos and TPP on field-collected varroa mites was insignificant (Table 1). Similarly, the dose–mortality regression lines for both treatments were equal (χ^2 = 1.58; df = 2; P = 0.45) and parallel (χ^2 = 1.06; df = 1; P = 0.30). In these tests, the enzyme carboxylesterase was not a major resistance factor in varroa mite populations of northern Florida.

The dose–mortality regression lines for coumaphos alone and coumaphos with the synergist DEM were equal (χ^2 = 1.81; df = 2; P = 0.91) and parallel (χ^2 = 0.13; df = 1; P = 0.71). The synergist ratio was 1.4-fold and was not statistically different from 1.0 (P > 0.05). Therefore, enhanced metabolism rates by glutathione S-transferases were not major factors of resistance in varroa mite populations of northern Florida (Table 1).

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Table 1. The toxicity of coumaphos with and without DEF (25 µg per vial), TPP (50 µg per vial), DEM (50 µg per vial), and PBO (50 µg per vial) to field-collected varroa mites.

Miticides	*N*[a]	Slope ± SE	LC50 (95% CL)[b]	SR (95% CL)[c]	χ^2
coumaphos	270	0.36 ± 0.15	0.0002 (0–0.0063)	—	2.3
coumaphos with DEF	273	0.22 ± 0.13	0.0014 (0–0.0314)	0.1 (0.02–1.72)	7.1
coumaphos	285	0.52 ± 0.20	0.0212 (0.0013–0.1614)	—	2.0
coumaphos with TPP	288	0.30 ± 0.15	0.0594 (0.0022–0.5341)	0.4 (0.06–1.89)	5.7
coumaphos	276	0.30 ± 0.14	0.0008 (0–0.0152)	—	11.3
coumaphos with DEM	279	0.38 ± 0.14	0.0594 (0.0022–0.5213)	1.4 (0.58–2.31)	3.1
coumaphos	315	0.53 ± 0.16	0.0082 (0.0002–0.0541)	—	3.7
coumaphos with PBO	318	0.38 ± 0.22	0.0001 (0–0.0022)	58.6 (43.36–75.35)	4.2

[a]Number of mites tested.
[b]Concentrations are expressed in µg per vial of the miticide tested.
[c]Synergism ratio (SR) calculated by dividing the LC50 for coumaphos alone by the LC50 for coumaphos with the synergist (DEF, TPP, DEM, or PBO).

Data indicated a significant increase of 58.6-fold in toxicity of coumaphos with the synergist PBO to varroa mites compared with coumaphos alone (Table 1). The LC50 of coumaphos alone compared with that of the coumaphos and synergist mixture was significantly different (Robertson & Preisler 1992). In addition, the dose–mortality regression lines for coumaphos alone and coumaphos with PBO were parallel (χ^2 = 0.29; df = 1; P = 0.59) but not equal (χ^2 = 9.33; df = 2; P = 0.009). These results suggest that mixed-function microsomal oxidases were major factors of resistance in varroa mite populations of northern Florida.

The toxicity (LC50) of the pyrethroid fluvalinate alone to varroa mites compared with fluvalinate with the synergist formamidine (Table 2) was significantly different. The dose–mortality regression lines for both treatments were parallel (χ^2 = 0.02; df = 1; P = 0.89) but not equal (χ^2 = 7.32; df = 2; P = 0.026). Data indicated a significant increase of 22.1-fold in synergism ratio with formamidine.

Similar differences were found with the organosphosphorus coumaphos alone and coumaphos with formamidine. Like with fluvalinate, the dose–mortality regression lines for both treatments were parallel (χ^2 =1.09; df = 1; P = 0.30) but not equal (χ^2 =6.32; df = 2; P = 0.042). The synergism ratio of 45.7-fold for coumaphos with formamidine was statistically different from 1.0 (P < 0.05).

The overall results indicated that both enhanced metabolism by mixed-function oxidases and target site insensitivity were the major factors of resistance in varroa mite populations collected in northern Florida. Target site insensitivity is consistent with Wang et al. (2002), who reported that mutations in a sodium channel gene were associated with target site resistance to pyrethroids in varroa mites. In the present study, measurements of esterase, carboxylesterase, and glutathion S-transferase failed to demonstrate that these factors were involved in resistance in varroa mites. However, Sammataro et al. (2005) suggested the possibility of esterase-mediated resistance mechanisms in varroa mite populations. Overall, our knowledge of these resistance mechanisms in varroa mites should provide useful insights in the development of a successful resistance management strategy, which could include the rotation of insecticides with differing mode of action and the use of synergists.

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Table 2. The toxicity of coumaphos and fluvalinate with and without formamidine (50 µg per vial) to field-collected varroa mites.

Miticides	*N*[a]	Slope ± SE	LC50 (95% CL)[b]	SR (95% CL)[c]	χ^2
Pyrethroid					
fluvalinate	315	0.47 ± 0.16	0.1503 (0.0012–0.0424)	—	2.9
fluvalinate with formamidine	297	0.51 ± 0.16	0.0504 (0.0021–0.3374)	22.1 (16.31–28.42)	1.3
Organophosphate					
coumaphos	282	0.26 ± 0.14	0.0365 (0.0013–0.2377)	—	6.7
coumaphos with formamidine	285	0.50 ± 0.18	0.0008 (0–0.0090)	45.7 (20.68–58.76)	5.1

[a]Number of mites tested.
[b]Concentrations are expressed in µg per vial of the miticide tested.
[c]Synergist ratio (SR) calculated by dividing the LC50 for fluvalinate or coumaphos alone by the LC50 for fluvalinate with formamidine or coumaphos with formamidine, respectively.

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Summary

The varroa mite (Acari: Mesostigmata: Varroidae) has developed resistance to the 2 major miticides (fluvalinate and coumaphos) registered for control of this invasive pest of bees (Hymenoptera: Apidae) in the United States. Comparative studies on miticide toxicity with and without the synergists piperonyl butoxide and formamidine indicated that enhanced metabolism by mixed-function oxidases and altered target site were the major mechanisms of resistance to organophosphorus and pyrethroid insecticides in varroa mite populations of northern Florida.

Key Words: honey bee; miticide; synergist; mixed-function oxidase; altered target site

Sumario

El ácaro *Varroa* (Acari: Mesostigmata: Varroidae) ha desarrollado resistencia a 2 acaricidas principales (fluvalinato y cumafós) registrados para el control de esta plaga invasora de las abejas (Hymenoptera: Apidae) en los Estados Unidos. Estudios comparativos sobre la toxicidad de los acaricidas con y sin las sinergistas butóxido de piperonilo y formamidina indicaron que el aumento del metabolismo por oxidasas de función mixta y sitio del enfoque alterado fueron los principales mecanismos de resistencia a los organofosforados y piretroides en poblaciones de ácaros *Varroa* del norte de Florida.

Palabras Clave: abejas; acaricidas; sinergistas; oxidasas de función mixta; sitio del enfoque alterado

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