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Original article

Control of *Varroa jacobsoni* Oud. resistant to fluvalinate and amitraz using coumaphos

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Abstract – We conducted laboratory tests investigating the mortality of mites infesting honey bee colonies in Minnesota after exposure to fluvalinate, amitraz, or coumaphos. Results indicated that these mites were not only resistant to fluvalinate, but also to amitraz. Coumaphos was effective against these resistant mites. A separate field trial was conducted with the same hives as those used in the laboratory test. The field trial supported the laboratory results, showing that mites in Minnesota are resistant to both fluvalinate and amitraz, and that coumaphos is effective in controlling these mites. This represents the first known reported case of amitraz resistance in *Varroa jacobsoni* in the U.S.A.

***Varroa jacobsoni* / resistance / fluvalinate / coumaphos / amitraz**

1. INTRODUCTION

In 1987, the honey bee parasitic mite *Varroa jacobsoni* Oud. was discovered in the U.S.A. [1]. Since that time it has spread throughout the continental U.S. *Varroa jacobsoni* damages immature and adult honey bees (*Apis mellifera* L.) by feeding on bee hemolymph, thus greatly weakening or killing the bee. Until recently, *V. jacobsoni* was effectively controlled by the pyrethroid acaricide fluvalinate, formulated as the product Apistan®. In 1998, however, several studies described resistance by

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V. jacobsoni to fluvalinate in the U.S. [2, 4]. Fluvalinate resistance has previously been documented in Europe [8].

Because Apistan is the only generally registered compound for *V. jacobsoni* control in the U.S., the advent of resistance has become a serious problem. There exist, however, several compounds that either have been effective in the past but are no longer registered, or that show promise as a new product for *V. jacobsoni* control. Amitraz, a formamidine, is an example of the first category, which was effective in controlling *V. jacobsoni* in past years. The organophosphate coumaphos (known as Perizin in Europe) is an example of the second category, which has shown promise as a new acaricide in the U.S. for *V. jacobsoni* control [3] (several U.S. states have received temporary emergency federal approval for the use of coumaphos against *V. jacobsoni*). We thus initiated the present study to document fluvalinate resistance in a U.S. population of *V. jacobsoni,* and to investigate the respective efficacies of amitraz and coumaphos in controlling these fluvalinate-resistant mites.

2. MATERIALS AND METHODS

Varroa jacobsoni-infested honey bee hives used in this study were located in north central Minnesota, in apiaries where difficulties in controlling *V. jacobsoni* with Apistan were observed by the commercial beekeeper. Reference hives were located in extreme south Texas. Laboratory and field techniques had previously revealed that *V. jacobsoni* in these Texas hives were susceptible to fluvalinate [4].

A laboratory test of each pesticide at each location was conducted. A discriminating dose of each pesticide was determined using the method previously described by Elzen et al. [4]. Treatments were prepared by dissolving technical grade pesticide in acetone and pipetting dilutions of the candidate pesticide into individual 20-mL glass vials. Vials were rolled on their sides until the acetone evaporated, leaving a uniform film of pesticide on the inner surface of the vials. Technical grade fluvalinate (98% purity) was obtained from Chem Service, Inc. (P.O. Box 599, West Chester, PA 19381–0599, U.S.A.); technical grade coumaphos (96% purity) was obtained from Bayer Corp. (8400 Hawthorn Rd., P.O. Box 4913, Kansas City, MO 64120–0013, U.S.A.); and technical grade amitraz (99% purity) was obtained from AgrEvo Co. (Little Falls Center One, 2711 Centerville Rd., Wilmington, DE 19808, U.S.A.).

In Texas, fluvalinate-susceptible *V. jacobsoni* were removed individually from bee brood cells and placed in pesticide-treated vials. Three adult female *V. jacobsoni*, removed from non-pigmented bee larvae/pupae, were placed in each vial and held for 24 h at 27 °C. Six h after initial exposure, a single bee pupa was added to each capped vial as a food source. An individual was considered dead after the treatment period if it showed no leg movement when gently prodded with a probe. Percentage mortalities resulting from various dilutions of each pesticide were used to calculate, using log-probit analysis [7], the single dose estimated to kill 80–90% of the susceptible Texas *V. jacobsoni*. This discriminating dose, a total of 33 treatment vials and 15 control vials (evaporated acetone only) were tested for each compound examined in Texas. In Minnesota, 4 colonies were chosen from a representative apiary and 17 test vials were utilized for each colony; 12 control vial tests were run concurrently. Mortality in Texas and Minnesota was compared with an expected 80–90% mortality using chi-square analysis [9].

A field experiment was initiated in October 1998 in Minnesota to provide information on resistance/control of *V. jacobsoni* in a commercial setting. Formulated 10% amitraz strips, impregnated into plastic, were obtained from Y-Tex Corp (1825 Big Horn Ave., Cody, WY 82414, USA).

Formulated 10% coumaphos strips, impregnated into plastic, were obtained from Bayer Corp. Formulated 10% fluvalinate strips, impregnated into plastic, were obtained from Dadant & Sons (51 S. 2nd St., Hamilton, IL 62341, U.S.A.). Two strips of the treatment type were inserted into the brood chamber and a sticky board was inserted under a screen on each hive bottom. Colonies averaged 10 frames of bees with less than one-half frame of brood, typical of hive strength during cooler temperatures in October. Seventeen colonies for each treatment type and 17 control colonies were tested. *V. jacobsoni* drop was counted 24 h later. Strips were left in place for 5 weeks and then were removed and a single fresh strip of coumaphos was inserted into each hive to obtain final mite knock-down. The resulting percentage change of initial versus final *V. jacobsoni* counts for each acaricide was compared with previously determined percentage change in completely susceptible mite populations [3] by chi-square analysis [9].

3. RESULTS AND DISCUSSION

Laboratory results of lethal dose estimates are shown in Table I. Fluvalinate was found to be the most toxic to Texas susceptible *V. jacobsoni* when comparing LD_{50}s. Amitraz was the least toxic, with 16.35 μg necessary for 50% mortality. In field studies on varying strengths of formulated coumaphos, a low concentration (1.0%) caused only 48% reduction of mites [3]. A low concentration of Apistan (2.5%), however, is quite effective in controlling *V. jacobsoni* in a field situation (Apistan product information guide), thus correlating with our results that fluvalinate is more toxic than coumaphos. Additionally, pyrethroid pesticides show very rapid toxicity effects, also perhaps explaining why fluvalinate was so much more toxic than coumaphos within the 24-h test period. We chose not to extend the treatment time in this study beyond 24 h, however, in an effort to minimize control mortality. The toxicity of coumaphos was additionally found to be within the toxicity range of organophosphates tested on at least one other susceptible arthropod adopting the same methods as those used in this study [6]. The relatively low lethality of amitraz observed in this study was also consistent with general reports that the biological action of amitraz is due more to its sublethal effects, rather than to a direct lethality [5].

Comparisons of mortalities at the LD_{90} values for each compound determined for the Texas (susceptible) *V. jacobsoni*

Table I. Lethal dose (LD) estimates for three acaricides on susceptible (Texas) *V. jacobsoni*.

Treatment	Lethal dose (90% CI)*		
	LD_{10}	LD_{50}	LD_{90}
Fluvalinate	0.13 (0.03–0.25)	0.56 (0.31–0.82)	2.38 (1.42–8.18)
Coumaphos	0.74 (0.03–2.19)	6.26 (2.06–11.31)	52.81 (26.42–287.83)
Amitraz	2.16 (0.01–8.31)	16.35 (1.44–34.93)	123.51 (57.14–1661.36)

* μg pesticide per inner surface of a 20-mL glass scintillation vial; CI: confidence interval.

Table II. Laboratory mortality of *V. jacobsoni* using LD_{90} estimates of three acaricides determined for susceptible (Texas) mites.

Treatment	Location	Mean % mortality
Fluvalinate	Texas	80.8
	Minnesota	14.2*
Coumaphos	Texas	–
	Minnesota	82.8
Amitraz	Texas	85.4
	Minnesota	32.3*

* Denotes significant difference compared to expected 80–90% mortality ($P < 0.05$; chi-square).

are shown in Table II. Whereas Texas *V. jacobsoni* showed 80–90% mortality for all compounds tested, Minnesota mites tested at the same doses as those used on the susceptible Texas mites showed significantly less mortality in amitraz and fluvalinate results (coumaphos was not tested on Texas *V. jacobsoni* because it was found to be effective on fluvalinate-resistant mites in Minnesota; hence, it would be equally effective on fluvalinate-susceptible *V. jacobsoni* in Texas). Only 32.3% mortality was seen in Minnesota *V. jacobsoni* exposed to amitraz, compared to an expected mortality of 80–90%. This is the first reported case of *V. jacobsoni* resistance to amitraz in the U.S. Additionally, only 14.2% mortality of Minnesota *V. jacobsoni* tested with the dose that caused 80–90% mortality of susceptible Texas mites was found when exposed to fluvalinate. Control mortality was an average of 6.1%, which was not used to adjust treatment mortalities. These laboratory findings indicate that the Minnesota population was resistant to both fluvalinate and amitraz. In contrast, 82.8% mortality found in the laboratory test of coumaphos for mites from Minnesota did not significantly differ from the expected 80–90% mortality. Coumaphos thus appears effective in killing fluvalinate- and amitraz-resistant *V. jacobsoni*.

Field studies further supported the laboratory findings (Tab. III). There was an increase of 89.1% over the treatment period for colonies exposed to Apistan, indicating little/no control, and thus very high resistance. The control obtained with amitraz was only 75.4%, which significantly differed from the expected 98% control using the same strip form of amitraz as used in previous studies in Guatemala [3]. Coumaphos was highly effective on these fluvalinate- and amitraz-resistant *V. jacobsoni* in Minnesota, providing 97.0% control over the treatment period.

It can thus be concluded that coumaphos strips can provide excellent control of *V. jabobsoni*, including those populations that are resistant to fluvalinate and amitraz. We are currently investigating the possibility of devising a resistance management plan for *V. jacobsoni* in the U.S.A. Such a plan would be based on the instability of fluvalinate resistance in *V. jacobsoni*; successful use of Apistan may be reintroduced after an interval with no Apistan exposure. We have verified that fluvalinate resistance in *V. jacobsoni* is an unstable trait (Elzen, unpubl. results), but how long the non-exposure period will be remains to be determined.

Table III. Change in population numbers over a treatment period for Minnesota *V. jacobsoni* using three acaricides.

Treatment	Mean % change over the treatment period
Fluvalinate	+ 89.1 *
Coumaphos	– 97.0
Amitraz	– 75.4 *
Control	+ 94.0 *

* Denotes significant difference compared to expected change of greater than 95% reduction ($P < 0.05$; chi-square).

Résumé – Contrôle, à l'aide du coumaphos, de *Varroa jacobsoni* Oud. résistant au fluvalinate et à l'amitraz. L'acarien parasite *Varroa jacobsoni* Oud. cause de gros dégâts aux populations d'abeilles domestiques (*Apis mellifera* L.) depuis son introduction aux Etats-Unis en 1987. Il n'existe actuellement qu'un seul produit autorisé aux États-Unis pour lutter contre *V. jacobsoni* : le fluvalinate (Apistan®). Nous avons effectué des tests sur le terrain et au laboratoire pour déterminer la sensibilité de *V. jacobsoni* à trois pesticides. un pyréthrinoïde, le fluvalinate, une formamidine, l'amitraz, et un organophosphoré, le coumaphos. Une résistance significative au fluvalinate été trouvée : la mortalité de l'acarien n'a été que de 14,2 % au cours du test en laboratoire et la population d'acariens s'est accrue de 89,1 % pendant la période de traitement au cours du test au champ. Une résistance significative de cette population à l'amitraz a été également trouvée : 32,3 % de mortalité au laboratoire et 75,4 % au champ. C'est le premier cas de résistance de *V. jacobsoni* à l'amitraz mentionné aux États-Unis. Le coumaphos s'est montré très efficace pour lutter contre les acariens devenus résistants au fluvalinate et à l'amitraz : la mortalité a atteint 85,4 % dans le test de laboratoire et la population au champ a été réduite de 97 % après traitement

***Varroa jacobsoni* / résistance / fluvalinate / amitraz / coumaphos**

Zusammenfassung-Bekämpfung von fluvalinat – und amitrazresistenten *Varroa jacobsoni* Oud. mit Coumaphos. Die Empfindlichkeit von *Varroa jacobsoni* gegen drei Pestizide wurde in Labor- und Freilandtests untersucht. Hierbei wurde eine signifikante Resistenz gegen Fluvalinat festgestellt. Dies ist die einzige in den U.S.A. zugelassene Substanz zur Bekämpfung von *V. jacobsoni*. Die Milbensterblichkeit in den Labortests betrug nur 14,2 %, das Populationswachstum der Milben im Freilandtest 89,1 %. In der gleichen Population wurde zusätzlich eine signifikante Resistenz gegen das Formamidin Amitraz festgestellt, die Mortalität im Labortest betrug nur 32,2 %, die im Feldtest nur 75,4 %. Dies ist der erste in den U.S.A. bekanntgemachte Fall von Resistenz von *V. jacobsoni* gegenüber Amitraz. Hingegen zeigte sich das Organophosphat Coumaphos als sehr effektiv zur Behandlung gegen fluvalinat- und amitrazresistente Milben und bewirkte eine Mortalität von 85,4 % im Labortest und einer Verminderung der Milbenpopulation um 97 % im Feldtest.

***Varroa jacobsoni* / Resistenz / Fluvalinat / Coumaphos / Amitraz**

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