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Detection of resistance in US *Varroa jacobsoni* Oud. (Mesostigmata: Varroidae) to the acaricide fluvalinate

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Abstract – Populations of *Varroa jacobsoni* Oud. from three geographic regions of the United States were assayed for resistance to fluvalinate. A bioassay using a discriminating concentration estimated to cause approximately 80–90 % mortality in a fluvalinate-susceptible strain of *V. jacobsoni* was used to compare percent mortalities between locations. An average of 73.3 % mortality was seen in two additional operations in Texas, not significantly less than the susceptible strain. An average of 23.7 % mortality was found for mites tested in Florida that had originated from field control failures; Florida test mortality was significantly less than that of the susceptible strain. An average of 65.1 % mortality was found for mites tested in California, also significantly less than mortality of susceptible mites. However, no field control failures were reported for the mites tested in California.

***Varroa jacobsoni* / fluvalinate / resistance /US / honey bee / *Apis mellifera* / Apistan®**

1. INTRODUCTION

The mite *Varroa jacobsoni* Oud. has become a serious parasite of the honey bee, *Apis mellifera* L., throughout most of the world. From its first discovery in the United States in 1987 [1], it has caused a dramatic decline of feral honey bee colonies and weakening of commercial apiaries that are not treated as needed [5].

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The only product currently registered in the US for *V. jacobsoni* control is a pyrethroid, fluvalinate (Apistan®). Plastic strips impregnated with 10 % fluvalinate are placed in bee hives and left for a period of 6–8 weeks. Bees come in contact with the strips and spread the acaricide throughout the colony. Mites are thus exposed to residual concentrations of fluvalinate.

Fluvalinate resistance in *V. jacobsoni* has been documented recently in Europe [9, 15]. Apistan® has been used in Europe for approximately 10 years, the same time period it has been used in the US. Baxter et al. [2] and Pettis et al. [11] have reported apparent cases of fluvalinate resistance in the US. We initiated this study to assess current susceptibility of *V. jacobsoni* to fluvalinate in several regions of the US.

2. MATERIALS AND METHODS

2.1. Mites

Honey bee colonies from each location were preliminarily examined for the presence of mites using the ether roll technique [4]. Frames of infested bee brood were collected and taken to a temporary laboratory at each location. Adult mites were collected by manually opening individual bee brood cells and removing adult mites from larvae and non-pigmented pupae with a water-moistened paint brush. Mites were not taken from adult bees in order to minimize mite mortality in the untreated control vials [10].

2.2. Chemical

Technical grade fluvalinate (98 % purity) was obtained from Chem Service, Inc., West Chester, PA, USA. Stock solution was made by dissolving 10.5 mg fluvalinate in 10.5 mL HPLC grade acetone. Dilutions of this stock were used in tests. All solutions were stored at –5 °C in darkness.

2.3. Assay vials

A glass vial residual bioassay was used to measure susceptibility of mites to fluvalinate [12]. Solutions containing known quantities of fluvalinate in acetone were pipetted into 20 mL borosilicate glass scintillation vials. Sufficient acetone was then pipetted into these vials to bring all volumes up to 0.5 mL acetone total. Control vials contained 0.5 mL acetone only. All vials were then rolled on their sides to evaporate the acetone and leave a film of fluvalinate residue on the inner surfaces. Rolling of vials assured a uniform distribution of pesticide on vial inner surfaces as demonstrated by repeatability of results obtained by the developers of this technique [12]. Additionally, the vial exposure method has been used extensively to detect and monitor pesticide resistance in other arthropods [6, 7, 13].

2.4. LC_{90} estimation

Mites from a south Texas bee operation were used as the reference susceptible strain from which a LC_{90} (lethal concentration) was estimated. This Texas operation did not receive Apistan® treatment in the preceding year. Preliminary field studies with these mites earlier indicated that excellent control of mites could be achieved with Apistan®: a ten-fold drop of mites onto sticky boards in hives treated with Apistan®, compared with untreated controls, was achieved. Four concentrations of technical grade fluvalinate in acetone plus a control were assayed: 0.3, 0.5, 1.0 or 3.0 μg in 0.5 mL acetone per vial. One mite per capped vial was tested, with five replications per dose. The entire assay was repeated five times, for a total of 125 mites. Tests were conducted at 26 ± 2 °C. Mite mortality was evaluated at 24 h. A mite was considered dead if no leg movement occurred when prodded with a blunt probe. De Guzman et al. [3] reported that mites were able to survive an average of 35.3 h on non-bee substrates. However, they considered a mite dead if it still showed leg movement but was uncoordinated; we considered such a mite as alive. The LC_{90} was estimated by probit analysis, taking into account natural mortality in the regression computing the LC_{90} [8]. The estimated LC_{90} concentration of 2.4 μg fluvalinate in 0.5 mL acetone per vial was used as the discriminating concentration in all subsequent trials.

2.5. Comparison of mortality between geographic locations

Mites from several geographic areas of the US were bioassayed to compare fluvalinate sus-

ceptibility. Two Texas locations were selected, excluding the Texas operation used as the susceptible reference strain. One of these Texas strains was evaluated for homogeneity of mortality response within a fluvalinate-susceptible operation: mites from three hives within the operation were tested using the vial method and proportions of dead mites were analyzed by chi-square analysis [14].

Six operations were chosen in Florida where mite control problems had been reported. In California, mites from seven operations were tested. Mite control problems with fluvalinate have not been reported in California; hives in which we discovered mites were missing Apistan® strips. All tests were conducted December 1997–March 1998.

Mites were assayed at the discriminating LC_{90} concentration of 2.4 µg fluvalinate in 0.5 mL acetone per vial using the bioassay described above, with the exception that three mites per capped vial were utilized. Each vial was replicated 30–33 times for a total of 90–99 mites from each operation. Three mites per control capped vial were replicated five times for a total of 15 control mites per location. Relative humidity in the temporary laboratory ranged from 40 to 62 %; capping of vials prevented loss of moisture. Mites in vials were held at 26 ± 2 °C and checked for mortality at 24 h. Resulting mortalities were pooled from each US region and were analyzed using chi-square analysis [14].

3. RESULTS

A value of 2.4 µg fluvalinate per vial (95 % confidence interval = 1.4–8.2 µg) was estimated to cause 90 % mortality in susceptible mite populations. Actual mortality of susceptible mites from the reference colony exposed to 2.4 µg was 80.3 %. The difference in actual (80.3 %) versus predicted (90 %) mortality of susceptible mites is due to the fact that lethal concentrations derived from probit analysis are estimates. Actual mortality can be expected to vary.

Results of the Texas locations tested showed 73.3 % mortality, which was not significantly different ($P > 0.05$) than the reference susceptible strain (*table I*). Response of mortality within a fluvalinate-susceptible operation (Texas) was homogeneous for all hives tested (*table II*). This homogeneity of response has been seen in additional susceptible operations tested (Elzen, unpublished data).

Comparison of mortalities of mites exposed to 2.4 µg of fluvalinate from Florida and California locations showed significant differences (*table I*). The proportion of mites from Florida operations significantly differed ($P < 0.05$) in mortality (23.7 %) from the susceptible reference mite population (80.3 %). California mite populations exhibited an intermediate response with an average of 65.1 % mortality (significantly less [$P < 0.05$] mortality than the susceptible reference strain). The average untreated control mortality for all tests during the experiment was 13.8 % (± 6.7 %).

Table I. Mean percent mortality of *V. jacobsoni* from three US regions, using a discriminating concentration of fluvalinate.

Location	n	Mean percent mortality ($\bar{x}$ ± SD)
Reference susceptible	99	80.8 ± 26.4
Texas	198	73.3 ± 21.3
Florida	585	23.7 ± 21.3*
California	639	65.1 ± 12.9*

* Significantly different when compared to proportion of dead mites of susceptible strain ($P < 0.05$, chi-square analysis).

Table II. Test of homogeneous response of susceptible mites to fluvalinate exposure, using a discriminating concentration of fluvalinate.

Hive no.	Mean percent mortality ($\bar{x}$ ± SD)
1	72.7 ± 21.2
2	80.4 ± 16.9
3	76.5 ± 22.9

No significant differences compared to susceptible reference strain (chi-square analysis, $P > 0.05$).

4. DISCUSSION

The results of this study support beekeeper reports of fluvalinate ineffectiveness in Florida mite populations. Resistance to fluvalinate in Florida mite populations appears to be the cause of mite control failures. Previous studies did not find quality defects in manufactured Apistan® strips [2].

The moderate level of resistance in California mite populations has not resulted in field control failures of fluvalinate as a miticide. However, concern about future effectiveness of fluvalinate is warranted.

From these results the need for an alternative class of miticide for mite control is evident. The development and registration of additional miticides is critical to the continued success of US beekeepers. Fluvalinate-resistant mites will probably spread owing to movement of bees. The continued use of a single miticide (fluvalinate) will continue to exert a strong selection pressure for fluvalinate resistance in mite populations.

Résumé – Détection de la résistance de *Varroa jacobsoni* à l'acaricide fluvalinate aux États-Unis. La résistance de *Varroa jacobsoni* Oudemans (Acari, Varroidae) à l'acaricide fluvalinate a été mesurée sur des acariens provenant de trois régions des États-Unis : Texas, Floride et Californie. Un test d'exposition résiduelle a été utilisé, dans lequel du fluvalinate pur à 98 %, dissout dans de l'acétone, sert à enduire des flacons de verre de 20 mL. On évapore l'acétone et il reste un film de fluvalinate. Les acariens de chaque site ont été exposés à une concentration de fluvalinate calculée pour provoquer une mortalité estimée par ordinateur à 90 %; cette concentration est de 2,4 µg de fluvalinate par flacon. Le pourcentage réel de mortalité à cette concentration a été de 80,3 % chez les acariens d'une souche de référence sensible prise au Texas et qui avait été traitée auparavant par le fluvalinate avec un excellent résultat. Cette mortalité moyenne de 80,3 % est significativement supérieure ($p < 0,05$) i) à la mortalité moyenne (23,7 %) des *V. jacobsoni* testés en Floride, où des problèmes de traitement avaient été mentionnés, ii) à la mortalité moyenne (65,1 %) des acariens testés en Californie, où pourtant aucun échec de traitement n'avait été signalé. Elle n'est en revanche pas significativement supérieure à celle (73,3 %) obtenue lors de deux autres opérations de traitement au Texas (*tableau I*). Les résultats suggèrent que les échecs de traitement en champ par le fluvalinate en Floride sont dus à des *V. jacobsoni* résistants à l'acaricide. Ils indiquent aussi que des problèmes sont susceptibles de se produire à l'avenir en Californie. Il faut s'efforcer d'homologuer d'autres composés chimiques qui puissent être utilisés en alternance avec le fluvalinate dans la lutte contre *V. jacobsoni* aux États-Unis. © Inra/DIB/AGIB/Elsevier, Paris

Varroa jacobsoni* / fluvalinate / resistance / États-Unis / *Apis mellifera

Zusammenfassung – Entdeckung der Resistenz von *Varroa jacobsoni* Oud. (Mesostigmata: Varroidae) gegen das Akarizid Fluvalinat in den USA. Eine mögliche Resistenz gegen das milbengiftige Fluvalinat bei *Varroa jacobsoni* Oud. wurde bei Milben aus drei geographischen Regionen der Vereinigten Staaten von Amerika bestimmt. Ein Test zur Aufdeckung von Rückständen wurde angewendet, bei dem das Innere von 20 mL Glasgefässen mit in Aceton gelöstem, technischem Fluvalinat überzogen wurde. Nach Verdunstung des Acetons blieb ein Film Fluvalinat zurück. Milben aus jedem Gebiet wurden einer bestimmten Fluvalinatkonzentration ausgesetzt, die nach einer Computer Berechnung 90 % Absterben von Fluvalinat-empfindlichen Milbenpopulationen bewirkt; wir berechneten diese Konzentration auf 2,4 µg Fluvalinat pro Glas. Der aktuelle Prozentsatz

der Sterblichkeit bei dieser Konzentration war 80,3 % bei Milben von einer eindeutig empfindlichen Linie aus Texas, wo bisher eine ausgezeichnete Milbenkontrolle mit Fluvalinat gegeben war. Der Durchschnitt dieser Referenzlinie mit 80,3 % Sterblichkeit war signifikant höher als die durchschnittliche Sterblichkeit von getesteten Milben in Florida (23,7 %) und Milben in Kalifornien (65,1 %). Aus diesen Ergebnissen läßt sich schließen, daß der Mißerfolg der Feldbehandlung mit Fluvalinat in Florida auf Akarizid-resistente Milben zurückzuführen ist. Sie deuten zusätzlich darauf hin, daß in Kalifornien möglicherweise in der Zukunft Schwierigkeiten bei der Milbenkontrolle auftreten werden. Man sollte sich bemühen zusätzliche chemischen Verbindungen zuzulassen, die in einem Rotationsverfahren mit Fluvalinat eine Kontrolle von *V. jacobsoni* in den USA gewährleistet.

Varroa jacobsoni* / Fluvalinat / Resistenz / USA / *Apis mellifera

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