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Original article

**First detection of strains of *Varroa destructor*
resistant to coumaphos.
Results of laboratory tests and field trials**

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Abstract – The susceptibility of four populations of *Varroa destructor* to the organophosphorous acaricide coumaphos was studied. The mites were taken from apiaries kept in Lombardy (northern Italy) and tested with a laboratory assay on paraffin wax with known concentrations of coumaphos. The susceptibility of two populations to coumaphos was close to that of susceptible populations, indicated in the literature (LC_{50} at 24 hours: 12.6 $\mu\text{g/g}$); a third population showed a significant, but slight increase in tolerance (LC_{50} at 24 hours: 29 $\mu\text{g/g}$) while the fourth population had a much higher LC_{50} (>200 $\mu\text{g/g}$ at 24 hours). In subsequent field trials carried out in the apiary in which the latter population had been sampled, the total average efficacy of two treatments with Perizin® (the commercial formulation of coumaphos) in colonies without capped brood was 46% (28–88%).

coumaphos / *Varroa destructor* / resistance / bioassay / field trial

1. INTRODUCTION

The spread of *Varroa destructor* Anderson and Trueman 2000 mites resistant to fluvalinate (Lodesani et al., 1995) caused serious losses to beekeeping in Italy (Astuti et al., 1995) and other countries (Eischen,

1998; Elzen et al., 1998; Fernandez and Garcia, 1998) and pointed out the need for regular surveys of the susceptibility of *V. destructor* to the acaricides used.

Bioassays that make it possible to study the susceptibility of the mites to pyrethroid and organophosphorous acaricides in the

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laboratory (Milani, 1995; Milani and Della Vedova, 1996) are a suitable tool to achieve this objective. With this purpose, in 1999 we started a survey of *V. destructor* populations taken from colonies in Lombardy (northern Italy) to verify their susceptibility to coumaphos in the laboratory and to test the efficacy of Perizin[®] (the commercial formulation of coumaphos) in the field.

2. MATERIALS AND METHODS

2.1. Laboratory tests

The bioassays were carried out with coumaphos (phosphorothioic acid O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl)-O,O-diethyl ester; Bayer; 97% purity), tested on mites taken from four apiaries

(Fig. 1) in Lombardy (northern Italy). The hives of the apiaries CO and MI had received one or two treatments with coumaphos each year for the last five years, while those of the apiaries VA1 and VA2 had been treated with an experimental thymol gel formulation (Colombo and Spreafico, 1999) in the summer of 1998.

The combs sampled from apiaries MI and VA1 contained mainly spinning larvae (*l5*), while those taken from the apiary VA2 contained mainly pupae with white eyes (*pw*). Two different tests were carried out for the CO apiary, the first on mites mainly taken from *pw*, the second on mites from cells with pupae with dark eyes (*pd*).

The combs were taken from the hives in September and immediately transferred to the laboratory to collect the mites. The



Figure 1. Location of apiaries.

bioassay was carried out as described by Milani and Della Vedova (1996) and at the following concentrations of the active ingredient: 0, 2, 5, 10, 20, 50, 100 µg/g. Each series of concentrations was tested in three replications; in total, approximately 45 mites per concentration and origin were tested. Mites from *15*, *pw* and *pd* were assayed separately. Dead and paralyzed mites were counted at 4, 24 and 48 hours after the introduction into the capsule. The data was compared with that available for a susceptible *V. destructor* population (indicated as UD95) never treated with coumaphos (Milani and Della Vedova, 1996).

A further sample of the CO population (called COb) was assayed in the laboratory of the Dept of Plant Defense, Udine University, at the following concentrations of the active ingredient in paraffin wax: 0, 10, 20, 50, 100, 200, 500, 1000 µg/g of coumaphos. The mortality was compared with that of mites from colonies never treated with coumaphos in the vicinity of Udine (UD99), tested by using the same series of capsules. These experiments were carried out on *V. destructor* taken from both *pw* and *pd* for sample COb and on mites from *pd* for sample UD99.

2.2. Field trials

In autumn, 10 colonies without capped brood in the CO apiary were treated twice with Perizin® (Bayer) at an interval of seven days according to label instructions. Seven and 12 days after the second treatment with Perizin, the colonies were treated with a solution of oxalic acid: 30 g of dehydrated oxalic acid were dissolved in 1000 ml of water and 3–4 ml of the solution were sprayed on each comb side (Imdorf et al., 1997). All the treatments were applied at midday on sunny days when the air temperature was above 15 °C. The colonies were housed in hives provided with a removable bottom insert that was smeared with vaseline to collect mites.

After each treatment, dead mites that fell on the bottom boards were counted and the Perizin® efficacy was calculated according to the formula: percentage of mites killed with Perizin® / the total number of mites killed with Perizin® and oxalic acid.

2.3. Statistical analysis

The laboratory tests data was analyzed using probit transformation (Finney, 1949, 1971) as in Milani (1995), by using an Excel 4.0 spreadsheet (unpublished data). The fiducial limits were computed according to Fieller (1954) and can be considered equivalent to the confidence interval.

3. RESULTS

3.1. Laboratory tests

The LC₅₀ of the populations VA1 and VA2 was comparable to that of UD95 and to that obtained for the population UD99, which was never treated with coumaphos (Tab. I, Fig. 2). The MI population showed a significant, but slight increase in the LC₅₀; the fiducial interval does not overlap with that obtained using the UD95 population (Tab. I, Figs. 2 and 3). In the CO population a larger significant increase in the LC₅₀ for the mites both from *pw* and *pd* was registered. In the tests carried out in Milan on mites taken from *pw*, the LC₅₀ exceeded the highest concentration tested (100 µg/g).

The results of CO population were confirmed by the tests carried out in Udine: the LC₅₀ for the mites from *pw* was 332 µg/g at 24 hours and 262 µg/g at 48 hours (Tab. I). 100% mortality of mites from CO was not reached, even at the highest concentration (1000 µg/g). Furthermore, the regression lines of CO and UD95 populations deviate considerably from parallelism (Figs. 2 and 3), indicating that the variance of the distribution of the mortalities is different (the CO population shows a broader distribution).

Table I. Median lethal concentration (LC₅₀) and its fiducial limits in assays of Udine in 1995 and in the current assays.

Origin of mites	Honeybee stage	24 h		48 h	
		LC ₅₀	Fiducial limits	LC ₅₀	Fiducial limits
UD95*	<i>pw</i>	12.6	11.0–14.3	9.8	8.7–11.1
MI	<i>l5</i>	29.0	23.4–34.4	21.5	17.7–25.1
VA1	<i>l5</i>	15.1	12.3–17.6	6.4	2.3–9.9
VA2	<i>pw</i>	15.2	12.2–18.4	8.1	5.2–10.7
CO	<i>pw</i>	234.6	> 116.17**	554.5	> 126.3**
CO	<i>pd</i>	99.4	48.7–700.1	73.7	34.1–272.0
CO _b	<i>pw</i>	331.3	96.2–1.1 × 10 ⁸	262.1	76.3–3.5 × 10 ⁴
CO _b	<i>pd</i>	60.2	0.001–208.0	59.1	2.2–145.5
UD99	<i>pd</i>	13.0	7.6–18.5	12.2	7.9–16.6

* from Milani and Della Vedova (1996).

** Cf. Fieller (1954).

3.2. Field trials

The results of the treatments carried out on the 10 colonies are indicated in Table II. The average efficacy of the two treatments with Perizin[®] was 46%, the minimum 28% (colony No. 1) and the maximum was 88% (colony No. 3). The average infestation was 600 mites per colony.

4. DISCUSSION

Utilizing laboratory tests, we were able to detect a population (CO) of *V. destructor*

20–50 times less susceptible than the reference populations (UD95, UD99). The increased variability in the individual response of this *V. destructor* population may be explained by its heterogeneity, i.e. by the presence of both susceptible and resistant mites.

The field trials confirm that the CO population contains mites that are resistant to Perizin[®] applied at the label-recommended doses. This also indicates that the bioassay used in this study makes it possible to detect populations of *V. destructor* resistant to coumaphos and can be used to organize the

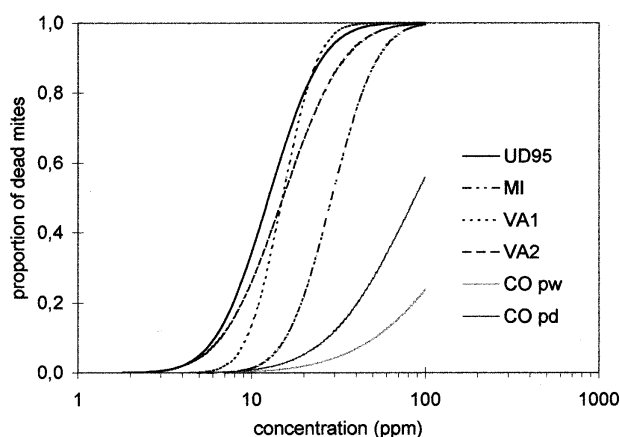


Figure 2. Proportion of dead *V. destructor* at 24 h in the assays carried out in Milano with coumaphos (regression lines are referred to strain Udine 95, considered normally susceptible).

Figure 3. Proportion of dead *V. destructor* at 24 h in the assays carried out in Udine with coumaphos (regression lines are referred to strain Udine 95, considered normally susceptible).

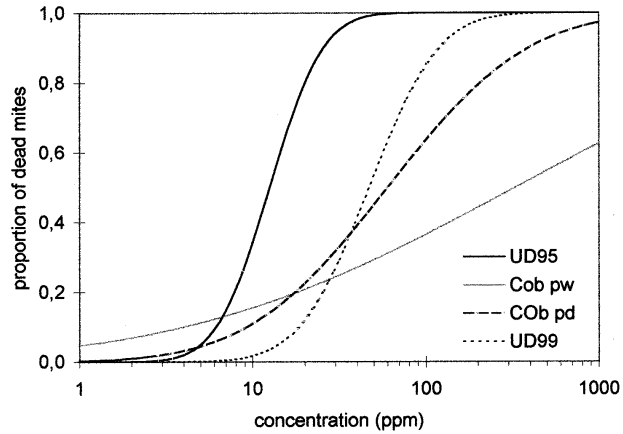


Table II. Field test results.

Hive	1	2	3	4	5	6	7	8	9	10	Average
Varroa mites killed by two treatments with Perizin [®]	278	106	449	346	366	504	162	169	56	305	274
Varroa mites killed by control treatments	711	69	61	53	708	843	177	88	125	428	326
Total mites killed	989	175	510	399	1074	1347	339	257	181	733	600
Perizin [®] efficacy	28	61	88	87	34	37	48	66	31	42	46

control of the mites at least on a regional scale.

Finally, this study further indicates the urgent need to expand and to implement more rational strategies in the fight against *V. destructor*. An integrated pest management approach needs to be developed to deal with the increasing problem of acaricide resistance in *V. destructor* populations worldwide.

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Résumé – Première mention de souches de *Varroa destructor* résistantes au coumaphos. Résultats de tests de laboratoire et d'essais au champ. En 1999 on a testé au laboratoire la sensibilité au coumaphos de la population de *Varroa destructor* (anciennement nommé *V. jacobsoni*) de quatre ruchers situés en Lombardie, Italie du Nord (Fig. 1). Les acariens ont été prélevés en septembre sur des rayons contenant (i) des larves L5 (stade de filage du cocon) pour les ruchers

MI et VA1, (ii) des nymphes *pw* aux yeux blancs pour le rucher VA2 et (iii) des nymphes *pw* et des nymphes *pd* aux yeux foncés pour le rucher CO.

Pour chaque population on a testé au laboratoire 45 acariens selon la méthode de Milani et della Vedova (1996), aux concentrations de coumaphos suivantes : 0, 2, 5, 10, 50 et 100 µg de coumaphos/g de cire de paraffine. Les résultats ont été comparés avec les données de la littérature pour une population sensible (UD95). Plus tard un test a été fait sur un autre échantillon de la population CO (Co_b) avec des concentrations de coumaphos de 0, 10, 20, 50, 100, 500 et 1000 µg/g et les résultats ont été comparés avec une population d'acariens issus du rucher UD95, qui n'avait jamais été traité au coumaphos. Les résultats ont été analysés à l'aide d'une transformation probit (Fieller, 1954 ; Finney, 1949, 1971 ; Milani, 1995). Dans les tests de laboratoire (Tab. I, Figs. 2 et 3) les concentrations léthales CL_{50} des populations VA1 et VA2 sont comparables à celles de la population de référence UD95 et à celles de la population UD99. Pour la population MI une augmentation significative mais légère de la CL_{50} a été observée. Le niveau de résistance de la population CO était beaucoup plus élevé et la CL_{50} 20 à 50 fois celle des populations sensibles. Dans la population CO, on n'a pas obtenu 100 % de mortalité des acariens au bout de 48 h, même en utilisant la forte concentration testée (1000 µg/g).

En se basant sur les résultats de laboratoire on a traité dix colonies sans couvain operculé du rucher CO deux fois au Perizin® (formulation commerciale du coumaphos) à sept jours d'intervalle selon les instructions portées sur le produit. Plus tard, les colonies ont été traitées deux fois avec une solution aqueuse d'acide oxalique pulvérisé sur les rayons. Les résultats des essais au champ (Tab. II) ont confirmé qu'au moins une partie de la population d'acariens pouvait résister à la dose recommandée de Perizin®. L'efficacité totale moyenne des deux traitements au Perizin® sans couvain a été de 46 % (minimum 28 %, maximum 88 %).

***Varroa destructor* / résistance / coumaphos / test biologique / essai au champ / acaricide**

Zusammenfassung – Erster Nachweis von Coumaphos-resistenten Populationen von *Varroa destructor*: Ergebnisse von Labor- und Freilandversuchen. Im Jahr 1999 wurde die Empfindlichkeit gegen Coumaphos bei 4 *Varroa destructor* Populationen in der Lombardei (Norditalien) im Labor untersucht (Abb. 1). Die Milben wurden aus Zellen mit Spinnmaden (*I5*) der Bienenstände MI und VA1, aus Zellen mit weißäugigen Puppen (*pw*) vom Bienenstand VA2 gesammelt, während vom CO Bienenstand Milben von weißäugigen und dunkeläugigen Puppen (*pd*) getrennt untersucht wurden.

Von jeder Population wurden 45 Milben mit einer Labormethode getestet, die von Milani und Della Vedova (1996) beschrieben wurde. Die Tests wurden mit folgenden Konzentrationen durchgeführt: 0, 2, 5, 10, 59, 100 µg Coumaphos/g Paraffinwachs. Die Ergebnisse wurden mit Daten für Coumaphos empfindlichen Populationen aus der Literatur (UD95) verglichen. Später wurde ein Test mit der CO Population (Co_b) mit Konzentrationen von 0, 10, 20, 50, 100, 200, 500, 1000 µg Coumaphos/g Paraffinwachs durchgeführt. Das Ergebnis wurde mit einer Milbenpopulation des Standes UD99 verglichen, der noch nie mit Coumaphos behandelt wurde. Die Ergebnisse wurden mit der Probit-Transformation (Fieller, 1954; Finney, 1949, 1971; Milani, 1995) analysiert.

In den Laborversuchen (Tab. I, Abb. 2 und 3) unterschied sich die LC_{50} weder von der Referenzpopulation UD95 noch von UD99. Bei der MI Population ließ sich eine leichte aber signifikante Erhöhung der LC_{50} beobachten. Das Niveau der Resistenz der CO Population war viel höher und die LC_{50} war 20–50 mal höher als die der empfindlichen Populationen. In der CO Population konnte nach 48 Stunden keine 100 % *Varroa* Sterblichkeit erreicht werden, auch nicht bei der

höchsten getesteten Konzentration von 1000 µg/g Coumaphos.

Auf Grund der Ergebnisse im Labor wurden 20 Bienenvölker ohne verdeckelte Brut im CO Bienenstand zweimal mit Perizin® (die kommerzielle Formulierung von Coumaphos) im Abstand von 7 Tagen nach der Gebrauchsanweisung behandelt. Später wurden die Völker 2mal mit einer wässrigen Oxalsäurelösung Wabe für Wabe gesprüht (Imdorf et al., 1997), Diese Feldversuche bestätigten, dass zumindest ein Teil der Milben die angegebene Dosis von Perizin® überlebte. Die durchschnittliche Wirksamkeit der beiden Perizin® Behandlungen in Völkern ohne Brut betrug 46 % (min. 28 % und max. 88 %).

Coumaphos / *Varroa destructor* / Resistenz / Biotest / Freilandversuch

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