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Original article

Decline in the proportion of mites resistant to fluvalinate in a population of *Varroa destructor* not treated with pyrethroids

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Abstract – The reversion of resistance to pyrethroids in *Varroa destructor* Anderson & Trueman was studied in Friuli (northern Italy), where resistance was detected in 1995 and pyrethroids had not been used since. Mites were sampled in seven localities each year between 1997 and 2000 and assayed in the laboratory for the resistance to fluvalinate by using paraffin coated capsules. Survival at the diagnostic concentration, expected to kill all susceptible mites (200 mg/kg), decreased in all the localities by about ten times in three years, from 19–66% to 1.3–7.8%. Thus, the disadvantage associated with the resistance to pyrethroids in *V. destructor* is small, as usual when resistance is due to monooxygenases. Its impact on the selection of resistant mites during annual application of treatments is negligible; appreciable effects of reversion can be expected only over many generations of the mite.

***Varroa destructor* / reversion / resistance / pyrethroids**

1. INTRODUCTION

In recent years, populations of *Varroa destructor* Anderson & Trueman resistant to pyrethroids have been detected in several countries (Lodesani et al., 1995; Trouiller, 1998; Elzen et al., 1998) and increasing attention has been paid to this problem. Several laboratory assays have been developed (e.g., Milani, 1995; Faucon et al., 1996; Colin et al., 1997; Elzen et al., 1998) and used to monitor the spread of resistant populations (Trouiller, 1998).

The biochemical mechanism of resistance has been investigated; monooxygenases of the P450 system are involved, at least in the strain of *V. destructor* that originated in Italy and later spread through the Old World, while esterases do not play a significant role (Hillesheim et al., 1996; Mozes Koch et al., 2000). Fast selection and spread of resistant mites make it unlikely that resistance is polygenic; this is a general rule with insects and mites (Roush and McKenzie, 1987).

Resistant genotypes usually are at some fitness disadvantage in the absence of pesticides (Roush and Daly, 1990; Denholm and Rowland, 1992), because of unbalanced or unregulated physiological processes. This makes the frequency of resistant mites decline when the acaricide is not used (a phenomenon usually called *reversion*). No information is available on the decrease in fitness associated with resistance to pyrethroids in *V. destructor*. More data on this aspect would be useful to elaborate theoretical models of the development of resistance and to develop integrated resistance

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management programmes (e.g., Denholm and Rowland, 1992).

The decrease in the fitness of resistant mites can be evaluated by determining fertility, fecundity, developmental time and other components of the fitness for both strains. However, small differences may be difficult to assess. Moreover, the fitness of a peculiar strain of mites may be influenced by other traits, casually associated with the genes for resistance.

For this reason, we preferred to follow changes in the percentage of resistant mites in natural populations not treated with pyrethroids over many generations (cf. Roush and McKenzie, 1987). We monitored levels of resistance in Friuli (north-eastern Italy), where resistance was detected in summer 1995, after the spread of the resistant strain to the remaining regions of Italy (Milani, 1996a; Trouiller, 1998), and pyrethroids were used for mite control for the last time in autumn 1995. This area was chosen since data on the treatments applied by beekeepers are reliable and introduction of *V. destructor* (due to migratory beekeeping) is minimal. The effect of initial, local inhomogeneities in the distribution of resistant mites (Milani, 1996a) was reduced by starting the survey two years after the detection of resistance. We tested mites with a laboratory assay at a fluvalinate concentration which discriminates resistant and susceptible genotypes; we determined also concentration-mortality curves at the end of the study period.

2. MATERIALS AND METHODS

Seven apiaries in different localities of the Friuli region were chosen (Fig. 1). These apiaries contained at least 20 colonies (in five apiaries there were more than 40 colonies). The apiaries were non migratory and at least 500 m away from other apiaries. In the area considered, colonies are broodless or almost broodless from mid October to February.

In these apiaries, treatments against *V. destructor* were carried out by beekeepers with products containing ethereal oils (Apilife Var®) and coumaphos. The same acaricides (plus oxalic acid, since 1998) were used by nearly all the beekeepers in the region; pyrethroids were not used in the years 1996–2000. In the resistant strain that originated in Italy, there is

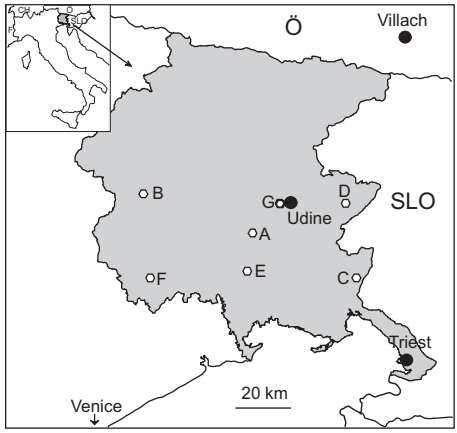

Figure 1. Location of the apiaries considered in the experiment.

no cross resistance between fluvalinate and coumaphos (Milani and Della Vedova, 1996).

In the years 1997–2000, in August or at the beginning of September, before autumn treatments, brood combs (at least two per apiary from different colonies) were taken and brought to the laboratory. Capped cells were opened, mites collected and assayed in capsules coated with paraffin containing 200 mg/kg fluvalinate; at this concentration, susceptible mites die, while most resistant mites survive (Milani, 1995; Trouiller, 1998). After 6 h, mites were transferred into Petri dishes with bee larvae and observed under a dissecting microscope at 6, 24 and 48 h; they were classified as mobile, paralysed (when they still moved some appendage but could not progress) and dead (when unable to move any part of the body) (Milani, 1995). As a rule, about one fourth of the mites from each sample were kept as controls and assayed in capsules without fluvalinate; fewer mites were used in control capsules, when the available sample was small, since the correction for natural mortality was not critical. Confidence limits were assigned to the proportion of mites dead at 200 mg/kg on the basis of the binomial distribution by means of a computer programme written for this purpose, and to the ratio of the proportions according to Nam (1995).

In 2000, assays at different concentrations were carried out on mites from the localities C, F and G (where enough mites were available), to obtain the concentration-mortality curve and compute the median lethal concentration, LC_{50}. For this purpose, besides the usual probit model, a modified probit model (Finney, 1971, Sect. 7.1 foll.) was used: the presence of resistant mites is accounted for, by

assuming that a proportion of mites survives at any dose, and so the parameter, *d*, sometimes called "natural immunity" was introduced, which was treated symmetrically to the natural mortality *c*. The "corrected mortality" fitted with the probit line was $m = (m_{obs} - c) / (1 - c - d)$, where m_{obs} is the observed mortality. Parameters of the probit regression were determined by maximizing the likelihood.

3. RESULTS

The number of mites found in the combs from a given apiary and available for the assays varied widely, depending on the treatments used by beekeepers, ranging from about 20 (in a single case) to over 500; on average, it was 160.

The mortality in controls was low (on average, 2.8%); variations among years and samples were small and not significant, with a single exception, where the mortality in the controls approached 9%. Thus, this case excepted, the average mortality in the controls was assumed as a joint estimate of the natural mortality to compute the so-called Abbott's correction (Tattersfield and Morris, 1924). Usually, the Abbott's correction affected the results by less than 1%.

In mites of the treated group (paraffin containing 200 mg/kg fluvalinate), the signs of intoxication (death and paralysis) varied with time as in previous tests (Milani, 1995); in particular, the percentage of paralysed mites (i.e., intoxicated but not dead) decreased with time and was only 2.3% at 48 h, making observations at this time the most reliable.

A reduction in the percentage of mites surviving at 200 mg/kg was observed in all the

Table I. Percent survival (corrected for natural mortality) of *V. destructor* mites at the diagnostic concentration (200 mg/kg) in each locality and sampling year; 95% confidence limits in parenthesis.

Locality	Year				1997/2000 ratio
	1997	1998	1999	2000	
A	32.8 (28–38)	32.0 (11–54)	(*)	7.0 (2.7–13)	4.7 (2.2–10)
B	39.4 (34–45)	38.4 (31–46)	11.2 (6–17)	4.4 (0.9–10)	8.9 (3.2–26)
C	42.3 (34–51)	14.6 (11–19)	10.4 (5–17)	1.3 (0.1–3)	33.1 (9.2–122)
D	66.2 (58–73)	51.3 (25–72)	13.1 (4–25)	3.3 (0.1–11)	19.8 (3.9–112)
E	18.6 (11–27)	13.0 (9–17)	11.8 (6–20)	3.1 (0.3–8)	6.0 (1.6–23)
F	55.0 (48–61)	44.3 (37–52)	29.1 (18–40)	7.8 (4.3–12)	7.1 (4.3–12)
G	40.9 (36–46)	13.1 (10–17)	7.7 (3.1–14)	5.1 (2.3–9)	8.1 (4.2–16)
Average	42.2	29.7	13.9	4.6	9.3

(*) data missing.

Table II. Median lethal concentration (LC_{50}) and its fiducial limits in four mite populations and reference data obtained in previous studies on different susceptible populations.

	C	F	G	Susceptible populations	
				Milani, 1995	Trouiller, 1998
LC_{50}	14	33	28	17	25
Fiducial limits	5–20	26–41	19–38	13–24	22–29
$p(\chi^2)$	0.18	0.62	0.07		
Estimated proportion of resistant mites ("natural immunity")	1.8%	7.7%	1.8%	–	–

localities, in each sampling year (Tab. I); the variation over three years was highly significant in each locality. On average, survival decreased about ten times, from 42.2% (range: 19–66%) in 1997 to 4.6% (1.3–7.8%) in 2000. The value 10 falls within the confidence limits of the ratio between the survival in 1997 and that in 2000 for all the localities.

Attempts to fit the concentration-mortality curve with the usual probit transformation lead to very highly significant χ^2; a better fit was obtained when the model was modified by introducing the "natural immunity"; the LC_{50} was in the range 14–33 mg/kg and the proportion of "immune mites" was close to that of mites surviving at the discriminant concentration of 200 mg/kg (Tab. II).

4. DISCUSSION

Low mortality in the controls demonstrated that increasing mortality in treated capsules is not due to causes other than the exposure to the fluvalinate.

Decreased survival at 200 mg/kg shows that the percentage of resistant mites decreases, since only 8% of resistant mites did not survive at this concentration (Trouiller, 1998), while susceptible mites died. No significant differences in the ratio between initial and final survival in different localities were observed. Immigration of susceptible mites may have contributed to this decrease, but the decrease took place in all the localities, even in those with the lowest initial proportion of resistant mites and continued for five years after the last application of pyrethroids.

Results obtained at different concentrations could not be fitted with a simple probit line, since the presence of a low proportion of resistant mites resulted in the occurrence of outliers rather than in a shift of a normal distribution, as a whole, towards higher tolerance. This makes parameters such as the LC_{50} less useful for characterizing mixed populations than survival at a diagnostic concentration. When the presence of resistant mites was accounted for by introducing a "natural immunity", a concentration-mortality curve for the susceptible part of the population was obtained. The LC_{50} calculated with this model showed only little variation with respect to the values previously obtained for susceptible populations (Milani, 1995; Trouiller, 1998); deviations may be explained by the natural variability among populations of *V. destructor* as well as the approximations introduced by this simplified model.

The percentage of resistant mites decreased by approximately ten times in three years, during which the mite performed over 30 generations. A slow decline indicates a small disadvantage associated with resistance to fluvalinate in *V. destructor*. This result is consistent with observations of a little or no disadvantage in insects, when resistance is due to monooxygenases. The decrease in fitness tends to be larger, when esterases are implied in the resistance mechanism, i.e., typically in resistance against organophosphates (in some strains or clones, resistant homozygotes appear to be only half as fit as susceptible genotypes) (Roush and Daly, 1990; further data and literature there). On the other hand, a previous study on fluvalinate resistant populations (Milani, 1996b) did not find morphological characters (such as smaller size, increased asymmetry) that indicate serious disadvantages. The decrease in the fitness of resistant homozygotes may differ from that of resistant heterozygotes (Roush and McKenzie, 1987), however, it has been noted that in natural populations of *V. destructor* inbreeding produces a very high proportion of homozygotes (Reich et al., 1998).

Owing to the large number of generations per year, over an eight-month broodright period, in *V. destructor*, a small decrease in fitness produced appreciable effects over several years. In areas where brood is present for longer times and thus the mite has more generations per year, reversion could be somewhat faster. Under the conditions of the present experiment, the effects produced in three years by the disadvantage associated with the resistance could not counterbalance the elimination of more than 90% susceptible mites caused by a single treatment with pyrethroids; of course, they have only a minor impact on the development of resistance if the treatments are repeatedly applied. In areas where strains of *V. destructor* resistant to pyrethroids are present, assuming a

tenfold decline in three years, treatments with these acaricides could be effective if used every 4–6 years and should be promptly followed by a further treatment to eliminate as many resistant mites as possible, before exchanges of mites with other colonies occur.

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Résumé – Baisse de la proportion d'acariens résistants au fluvalinate dans une population de *Varroa destructor* non traitée aux pyréthrinoïdes. Les insectes et les acariens résistants présentent généralement un certain désavantage de valeur sélective en l'absence de pesticides à cause de processus physiologiques déséquilibrés. Ce désavantage provoque une baisse de la fréquence des génotypes résistants lorsqu'il n'y a pas d'application de pesticides ; ce phénomène s'appelle la réversion et on peut l'exploiter dans des programmes de gestion de la résistance.
On a étudié la réversion de la résistance aux pyréthrinoïdes chez *Varroa destructor* Anderson & Trueman en surveillant la baisse de la proportion d'acariens résistants dans le Frioule (Italie du Nord), région où la résistance a été signalée en 1995 et où les pyréthrinoïdes n'ont plus été utilisés depuis cette date. Les acariens ont été prélevés dans sept localités (Fig. 1) chaque année, de 1997 à 2000, et testés au laboratoire à l'aide de capsules enduites de paraffine renfermant 200 mg/kg de fluvalinate ; à cette concentration les acariens sensibles meurent alors que plus de 90 % des acariens résistants survivent. La survie dans les diverses localités à la concentration diagnostic était comprise à l'origine entre 19 et 66 % et à la fin de la période d'étude entre 1,3 et 7,8 % (Tab. I). La baisse du pourcentage d'acariens résistants (correspondant à un facteur 10 environ en trois ans) a été observée dans toutes les localités. Les courbes de contentration-mortalité obtenues à la fin de la période d'étude dans trois localités sont celles auxquelles on s'attend pour une population comprenant une majorité d'acariens sensibles et une faible proportion (1,8 à 7,7 %) d'acariens résistants (Tab. II).
Le désavantage associé à la résistance est faible et n'a produit des effets qu'après de nombreuses générations de *V. destructor*. L'influence de la réversion sur la sélection d'acariens résistants est négligeable si les traitements ont lieu chaque année. Dans les régions où les acariens résistants sont présents, les traitements aux pyréthrinoïdes contre *V. destructor* ne peuvent être efficaces que s'ils sont appliqués à intervalles de plusieurs années.

***Varroa destructor* / réversion / résistance / pyréthrinoïde**

Zusammenfassung – Verringerung des Anteils von Fluvalinat-resistenten Milben in einer nicht mit Pyrethroiden behandelten Population von *Varroa destructor*. Resistente Insekten und Milben haben bei Abwesenheit der Pestizide normalerweise Fitnessnachteile, weil physiologische Prozesse aus dem Gleichgewicht geraten sind. Diese Nachteile führen zu einer Abnahme in der Häufigkeit der resistenten Genotypen, sobald das Pestizid nicht angewendet wird. Dieses Phänomen wird als Reversion (Umkehrung) bezeichnet und kann für Anwendungsprogramme zur Resistenzbeherrschung genutzt werden.
Die Reversion der Resistenz gegen Pyrethroide bei *Varroa destructor* Anderson & Trueman wurde durch Bestimmung der Abnahme des Anteils resistenter Milben in Friuli (Norditalien) untersucht. Dort waren 1995 Resistenzen nachgewiesen worden, seit dieser Zeit wurden keine Pyrethroide mehr angewendet. Die Milben wurden zwischen 1997 und 2000 jedes Jahr an 7 Orten (Abb. 1) gesammelt und im Labor in mit 200 mg/kg Fluvalinat versetzten Paraffinwachs ausgestrichenen Schalen getestet. Bei dieser Konzentration sterben nichtresistente Milben, während mehr als 90 % der resistenten Milben überleben. Am Anfang betrug bei dieser diagnostischen Konzentration die Überlebensrate bei den verschiedenen Proben 19–66 %. Am Ende der Testperiode waren es noch 1,3–7,8 % (Tab. I). Die Abnahme im Prozentsatz der resistenten Milben (etwa 10fach in 3 Jahren) wurde an allen 7 Orten beobachtet. Die Kurven der Sterblichkeit über der Konzentration zu Ende des Tests entsprechen an 3 Orten denen, die für eine Population erwartet wird, die in der Mehrzahl aus nicht resistenten Milben mit einem geringen Anteil (1,8–7,7 %) an resistenten Milben bestehen (Tab. II).
Die Nachteile, die mit der Resistenz verbunden sind, sind gering und hatten nur nach vielen Milbengenerationen einen annehmbaren Effekt. Der Einfluss der Reversion auf die Selektion von resistenten Milben ist unbedeutend, wenn jedes Jahr behandelt wird. In Gebieten, wo es resistente Milben gibt, können Behandlungen mit Pyrethroiden gegen *V. destructor* nur in Abständen von mehreren Jahren erfolgreich angewendet werden.

***Varroa destructor* / Reversion / Resistenz / Pyrethroide**

REFERENCES

Colin M.E., Vandame R., Jourdan P., Di Pasquale S. (1997) Fluvalinate resistance of *Varroa jacobsoni* Oudemans (Acari: Varroidae) in Mediterranean apiaries of France, Apidologie 28, 375–384.

Denholm I., Rowland M.W. (1992) Tactics for managing pesticide resistance in arthropods: theory and practice, Annu. Rev. Entomol. 37, 91–112.

Elzen P.J., Eischen F.A., Baxter J.R., Pettis J., Elzen G.W., Wilson W.T. (1998) Fluvalinate resistance in *Varroa jacobsoni* from several geographic locations, Am. Bee J. 138, 674–676.

Faucon J.P., Drajnudel P., Fléché C. (1996) Varroose : mise en évidence de la résistance du parasite aux acaricides par la méthode de « détermination du temps létal moyen », Apidologie 27, 105–110.

Finney D.J. (1971) Probit analysis, 3rd ed., Cambridge University Press, Cambridge.

Hillesheim E., Ritter W., Bassand D. (1996) First data on resistance mechanisms of *Varroa jacobsoni* (Oud.) against tau-fluvalinate, Exp. Appl. Acarol. 20, 283–296.

Lodesani M., Colombo M., Spreafico M. (1995) Ineffectiveness of Apistan® treatment against the mite *Varroa jacobsoni* Oud in several districts of Lombardy (Italy), Apidologie 26, 67–72.

Milani N. (1995) The resistance of *Varroa jacobsoni* Oud to pyrethroids: a laboratory assay, Apidologie 26, 415–429.

Milani N. (1996a) Die Ausbreitung apistanresistenter Varroamilben im nordöstlichen Italien, Bienenvater 117, 290–293.

Milani N. (1996b) Examination of the morphometry of populations of *Varroa jacobsoni* Oudemans resistant and susceptible to tau-fluvalinate, Redia 79, 47–56.

Milani N., Della Vedova G. (1996) Determination of the LC_{50} in the mite *Varroa jacobsoni* of the active substances in Perizin® and Cekafix®, Apidologie 26, 67–72.

Mozes Koch R., Slabezki Y., Efrat H., Kalev H., Kamer Y., Yakobson B.A., Dag A. (2000) First detection in Israel of fluvalinate resistance in the varroa mite using bioassay and biochemical methods, Exp. Appl. Acarol. 24, 35–43.

Nam J.-M. (1995) Confidence limits for the ratio of two binomial proportions based on likelihood scores: non iterative method, Biometr. J. 3, 375–379.

Reich S.E., Fuchs S., Schulz A., Urfer W. (1998) Geometric approximation of the infestation of honey bee brood cells by *Varroa jacobsoni* and implications for the estimation of brood infestation, for population models and for the proportion of non sibling matings, J. Apic. Res. 37, 115–123.

Roush R.T., McKenzie J.A. (1987) Ecological genetics of insecticide and acaricide resistance, Annu. Rev. Entomol. 32, 361–380.

Roush R.T., Daly J.C. (1990) The role of population genetics in resistance research and management, in: Roush R.T., Tabashnik B.E. (Eds.), Pesticide resistance in arthropods, Chapman and Hall, New York, pp. 97–152.

Tattersfield F., Morris H.M. (1924) An apparatus for testing the toxic values of contact insecticides under controlled conditions, Bull. Entomol. Res. 14, 223–233.

Trouiller J. (1998) Monitoring *Varroa jacobsoni* resistance to pyrethroids in western Europe, Apidologie 29, 537–546.