

RESISTANCE BY THE BLUE TICK (*BOOPHILUS DECOLORATUS*) TO THE SYNTHETIC PYRETHROID, FENVALERATE

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ABSTRACT

COETZEE, BRIDGETTE B., STANFORD, G. D. & DAVIS, D. A. T., 1987 Resistance by the blue tick (*Boophilus decoloratus*) to the synthetic pyrethroid, fenvalerate. *Onderstepoort Journal of Veterinary Research*, 54, 83-86 (1987).

The Shaw larval test, in conjunction with adult tick immersion and stall tests, was utilized to confirm that a field strain of *B. decoloratus*, from Natal, is highly resistant to the ixodicide fenvalerate (Factor of resistance 4 744). This resistance developed over a reported 18 months of usage for cattle dipping.

INTRODUCTION

Larval tests undertaken on a field strain of *Boophilus decoloratus*, submitted as part of the Company's resistance surveillance programme, indicated resistance to fenvalerate. Since this was considered to be the first verifiable indication in South Africa of resistance of a strain of ticks to a pyrethroid, it was decided to investigate the resistance status of this strain of tick to fenvalerate, and other pyrethroids, as well as certain organophosphates and amitraz. Accordingly, extensive *in vitro* and *in vivo* tests were carried out.

The work fell naturally into 2 parts: first, studies to confirm the resistance to fenvalerate and validate the larval test as a method of detecting resistance to pyrethroids, and second, the use of the larval and other tests to establish the resistance spectrum of the strain. Certain items in the second part were carried out simultaneously with corresponding items in the first part but are published separately.

MATERIALS AND METHODS

Ixodicides

1. Fenvalerate 10 % m/v emulsifiable concentrate (e.c.) prepared by Formulation and Application Research, Coopers Animal Health, Berkhamsted, UK.
2. Sumitik*, an e.c. containing 20 % m/v fenvalerate.

Tick species and strains

1. A strain of *B. decoloratus*, 'Pot-the-Red', known to be susceptible to organophosphates and never exposed to pyrethroid chemicals, held at Kwanyanga as a reference strain.
2. A strain of *B. decoloratus*, 'Braemar', suspected to be resistant to fenvalerate, obtained from Izingolweni District, Natal.

Experimental animals

Male Friesland calves (8 months of age) of similar conformation, obtained from 1 property.

General plan

Larval tests were carried out to find the range of concentrations of fenvalerate required to establish lethal concentration 99 % (LC 99 %).

Adult immersion tests were carried out to assess the activity of the ixodicide against the reproductive ability of the 2 strains. Stall tests were carried out to evaluate the activity of the ixodicide against all stages of both strains on infested calves.

Unfed larvae

The technique used was that described initially by Shaw (1966) and later modified to include a longer holding period for the larval ticks after treatment (Shaw, Cook & Carson, 1968). A further modification to this

technique was used in this work whereby 1 operator carried out the test in duplicate from a common reservoir of larvae, as distinct from 2 operators each conducting a test simultaneously.

Using the fenvalerate 10 % e.c., the susceptibility of the 'Braemar' strain was compared with that of the susceptible 'Pot-the-Red' strain. In order to obtain the most accurate regression line, larval tests on the 'Braemar' strain were carried out in 2 stages using a range of 14 fenvalerate concentrations instead of the usual 7. Because of the limitations in the number of concentrations which can be included in 1 test of this nature, the first stage included the use of 10 concentrations. This was immediately followed by the remaining 4, in the second stage.

Adult immersion test

Engorged female ticks, collected from donor animals on which the relevant strains had been raised, were divided into groups of 40, mass-measured as a group and placed in copper tubes (length 100 mm/diameter 40 mm) the lower ends of which were covered with fine-mesh copper netting.

A volume of 50 ml of wash per concentration was prepared and poured into 50 mm wide-mouthed glass jars. A range of 7 concentrations of the 10 % e.c., plus a water control, was used.

Because of the gross disparity in the susceptibility of the 2 strains, the definitive test was carried out on the 'Braemar' strain with a range of concentrations 10-fold in strength compared with those used on 'Pot-the-Red'.

The groups of 40 ticks, each group in its own container, were immersed for 10 min each in the appropriate wash. The copper tubes containing the ticks were gently shaken every 30 s to ensure good dispersion of the wash. After its removal from the wash each tube was placed upright on paper towelling for a few seconds to absorb any wash clinging to the copper mesh, and then suspended for 1 h in order to dry.

The groups of ticks were placed in series onto sheets of plastic laminate-covered plywood, each tick being stuck in its group, on its dorsum onto the upper side of double-sided sticky tape attached to the plywood. The front quarter of the body of each tick was allowed to project beyond the edge of the sticky tape to avoid eggs being laid onto the sticky tape. The strips of tape were arranged 4 cm apart and individual ticks were placed 2 cm apart. The boards holding the ticks were then placed in a constant environment room (25 °C, 85 % RH).

Examinations were made 21 days after treatment to determine inhibition of oviposition, calculated by comparing visually the size of the egg batches in the test group with those of the water control. The following scoring system was used:

- 100 % oviposition: 4 points
- 75 % oviposition: 3 points
- 50 % oviposition: 2 points
- 25 % oviposition: 1 point
- 0 % oviposition: 0 point

* Shell Chemical, South Africa (Pty) Ltd.

TABLE 1 Fitted parameter estimates, with ED50/ED99 values and 95 % fiducial limits and test for parallelism and factors of resistance

Variable	Strain	a	b	ED50	95 % fiducial limits	ED99	95 % fiducial limits	Test for parallelism	FOR	95 % fiducial limits
Larval kill	B	1.084	0.745	0.3974	0.5898	3.2924	1.9704	NS	4744	2478
	P	3.421	0.589	0.00003373	0.00009106	0.0004900	0.0002417			9836
Oviposition	B	0.516	0.519	3.3101	14.6693	69.1297	15.3255	NS	147	50.9
	P	1.256	0.341	0.04173	0.10590	4.2541	0.8671			627
Larval hatch	B	0.947	-0.322	3.1684	25.5563	0.02358	0.00049	NS	244	82.8
	P	0.037	-0.396	0.01296	0.02571	0.0002425	0.0000003			1499
Reproduction	B	0.779	0.567	1.0275	3.7484	16.6229	4.2776	NS	64.4	22.3
	P	1.782	0.553	0.01579	0.03687	0.2734	0.1153			361

Note: a and b above refer to the intercept and slope of the fitted line
 : All variables have been converted to arcsins
 B: Braemar
 P: Pot-the-Red
 NS: Not significant

Where necessary, the size of the egg batch in relation to the size of the tick was considered.

A corrected oviposition percentage was then calculated according to Abbott's formula, as described by Shaw.

$$\text{i.e. CM \%} = \frac{\%i - \%c}{100 - \%c} \times \frac{100}{1}$$

Where %i = % mortality in concentration i,
 %c = % mortality in water control.
 CM% = corrected mortality.

The masses of the batches of eggs of each group were determined collectively without being mixed. Then the egg batch of each tick was tubed separately in a glass vial with a cotton wool bung and replaced in the constant environment room for a further 35 days.

Fifty-six days after treatment each batch of eggs was examined to assess the percentage hatch of eggs, using the following scoring system:

Hatch rate %	Points
100	4
66-99	3
33-65	2
1-32	1
0	0

The estimated reproduction (ER) was calculated using the formula of Drummond, Ernest, Trevino, Gladney & Graham (1973), i.e.:

$$\frac{\text{Mass of eggs} \times \text{percentage hatch}}{\text{Mass of females}} = \text{ER}$$

Reproduction index (RI) which reflects the percentage of control of ER was determined by comparing the ER of treated ticks with that of untreated ticks, as under:

$$\frac{\text{ER (control)} - \text{ER (treated)}}{\text{ER control}} \times 100 = \text{RI}$$

Stall test

Two calves of approximately 160 kg live mass were placed in the Tick House at the Kwanyanga Research Station under conditions of naturally fluctuating temperatures and humidity. The calves were restrained in individual crates with head yokes to prevent self-grooming. Each crate was placed in its own moated, walled area, with the floor of the crate raised 40 cm from the floor of the moated area.

The calves were infested twice weekly for 3 weeks with approximately 1000 larvae (3-4 weeks old) of either the 'Pot-the-Red' or the 'Braemar' strains. Approximately 21 days after commencement of infestation (I+21) engorged female ticks began to drop from the calves. Pre-treatment collections of detached engorged female ticks were made on Day I+22 and I+23 in order to monitor oviposition and egg viability of the strains before treatment. They underwent the same collecting and incubating procedures described below for the post-treatment collections.

On Day I+24 the animals were handsprayed using a high pressure spray system at an operating pressure of 275 kPa using Sumitrik at 0.02 %, the concentration of fenvalerate specified by the makers. A volume of wash of 15 l was used for each animal and every effort was made to saturate the animals to skin level, utilising the standard technique customarily used at the Kwanyanga Research Station.

All ticks detaching between treatment (T) and T+1 h were collected and discarded. Thereafter daily collections were made in order to monitor oviposition and egg

TABLE 2 Summary of stall test results (pooled over days within pre- and post-treatment)

Compound	Strain	Pre-treatment			Post-treatment			P values for pre- vs post-comparisons for per cent:	
		No. females tubed	Per cent ovip.	Per cent hatch	No. females tubed	Per cent ovip.	Per cent hatch	Oviposition	Hatch
Fenvalerate	Pot-the-Red	65	100	89	423	58	33	<0,001	<0,001
Fenvalerate	Braemar	100	100	97	912	81	92	<0,001	<0,091

TABLE 3 Summary of stall test results (pooled over days within the post-treatment)

Compound	Pot-the-Red			Braemar			P values for pre- vs post-comparisons for per cent:	
	No. females tubed	Per cent ovip.	Per cent hatch	No. females tubed	Per cent ovip.	Per cent hatch	Oviposition	Hatch
Fenvalerate	423	58	33	912	81	92	<0,001	<0,001

viability. The ticks were not washed but when selecting the daily sample of engorged females for incubation, care was taken to separate each tick from any debris before it was tubed. Detaching ticks were counted, the numbers recorded and all ticks up to a maximum of 50 from each animal on each day were tubed. The labelled tubes were placed in a constant environment room (25 °C, 85 % RH), for 8 weeks after which they were examined and the oviposition and hatch percentage for each tick was calculated, the latter being calculated relative to the number of ticks ovipositing.

A sample of the fenvalerate wash used was submitted to Coopers Animal Health Laboratory, Berkhamsted, for chemical analysis.

Statistical analysis

The results were submitted to Coopers Animal Health, Berkhamsted, UK, for statistical analysis.

RESULTS

Larval and adult immersion tests

The following measurements were taken and subjected to statistical analysis:

- Larval kill (%)
- Inhibition of oviposition (%)
- Larval hatch (%)
- Estimated reproduction (%)

All percentage results, corrected for control, were transformed to the arcsin scale, and all concentrations transferred to logarithms (base 10).

The SAS statistical package was used to fit, for each variable above, a linear dose-response relationship for each strain separately. These models were then used to estimate ED50/ED99 values for percentage results with 95 % fiducial limits using a program written in APL.

A parallel-line fit was then derived for each variable, i.e. fitting a common slope to each strain and, where this was shown to be as adequate a model as separate lines, estimates of factors of resistance were derived with limits, again using a program written in APL.

Parameter estimates for the separate-line fits are given in Table 1, together with the derived ED50/ED99 values with limits. Tests for parallelism are also shown, together with factors of resistance, with limits.

The factor of resistance (FOR) of 4 744, obtained from the Shaw larval test, shows marked resistance to fenvalerate. A significant level of resistance was also demonstrated with regard to oviposition, larval hatch and reproduction.

Stall tests

The spraywash was shown to contain 0,02 % m/v fenvalerate.

Using the chi-squared test the results were analysed statistically and are presented in Tables 2 and 3.

The difference between the 2 strains with regard to the oviposition and hatch of eggs laid by engorged female ticks, collected from the cattle handsprayed with fenvalerate, was significant ($P < 0,001$), thereby confirming resistance in the 'Braemar' strain.

DISCUSSION

The results of the larval, adult immersion and stall tests were fully comparable with each other and each showed an extremely high level of resistance. The Shaw larval test is, therefore, shown to be a valid means of testing ticks for resistance to pyrethroids.

In the case of the larval and adult immersion tests, the concentration of ixodocidal wash required to achieve a kill of the 'Braemar' strain was of such an order that the possibility should not be discounted that some of the effect was due to the presence of excessive amounts of solvents, emulsifiers, etc.

The level of resistance shown by the 'Braemar' strain to fenvalerate (FOR 4 744 at the LC 99 % level) is such that complete lack of control of this tick strain, in a field situation, could be expected. This confirmed reports from the field that short interval dipping (5, 5 and 4 days interval) in 'Sumitik', at the manufacturer's recommended usage concentrations, had failed to effect control. What is considered of importance with regard to cattle tick control in South Africa is the fact that this resistance developed during an 18-month period of use of fenvalerate. A similarly rapid development of resistance to an ixodocide has previously only been observed in *B. decoloratus* following the use of BHC. The resistance spectrum of the 'Braemar' strain to other ixodocides will be presented in a subsequent publication. The indications, however, do not rule out the possibility that cross-resistance to organochlorines may play a role in the development of resistance to pyrethroids.

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REFERENCES

- SHAW, R. D., 1966. Culture of an organophosphorus-resistant strain of *Boophilus microplus* (Can.) and an assessment of its resistant spectrum. *Bulletin of Entomological Research*, 56, 389-405, Fig. 1-4.

RESISTANCE BY THE BLUE TICK TO THE SYNTHETIC PYRETHROID, FENVALERATE

SHAW, R. D., COOK, M. & CARSON, R. E., 1968. Developments in the resistant status of the southern cattle tick to organophosphorus and carbamate insecticides. *Journal of Economic Entomology*, 61, 1590-1594.

DRUMMOND, R. O., ERNEST, S. E., TREVINO, J. L., GLADNEY, W. J. & GRAHAM, O. H., 1973. *Boophilus annulatus* and *B. microplus*: Laboratory tests of insecticides. *Journal of Economic Entomology*, 66, 130-133.