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THE RESISTANCE SPECTRUM SHOWN BY A FENVALERATE-RESISTANT STRAIN OF BLUE TICK *(BOOPHILUS DECOLORATUS)* TO A RANGE OF IXODICIDES

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ABSTRACT

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A strain of *Boophilus decoloratus*, resistant to fenvalerate, was subjected to larval immersion, adult immersion and stall tests using the following classes of ixodicides: organochlorines, organophosphates, a diamidide and pyrethroids. A susceptible reference strain of *B. decoloratus* was used for comparative purposes. The results indicated a high level of resistance to DDT and camphechlor, slight tolerance to dioxathion, chlorfenvinphos and pirimiphos ethyl, full susceptibility to bromophos ethyl and amitraz, but marked resistance to cyhalothrin, cypermethrin, deltamethrin and flumethrin. This marked resistance in the strain therefore appears to be widespread within the pyrethroid group of chemicals and may have developed as a result of organochlorine cross-resistance.

INTRODUCTION

The resistance of the 'Braemar' strain of *Boophilus decoloratus* to the synthetic pyrethroid, fenvalerate, has recently been established (Coetzee, Stanford & Davis, 1987). During the previous investigations the opportunity was taken to determine the resistance spectrum of the 'Braemar' strain to other pyrethroids, certain organophosphates, a diamidide and some organochlorines.

MATERIALS AND METHODS

Ixodicides

The following experimental formulations were prepared by Formulation and Application Research, Coopers Animal Health, Berkhamsted.

Dioxathion 20 % m/v emulsifiable concentrate (e.c.)
Camphechlor 75 % m/v e.c.
Chlorfenvinphos 20 % m/v e.c.
Supamix 20 % m/v e.c. (a mixture of chlorfenvinphos and dioxathion)
Bromophos ethyl 20 % m/v e.c.
DDT 20 % m/v e.c.
Deltamethrin 10 % m/v e.c.
Cypermethrin 10 % m/v e.c.
Amitraz 20 % m/v e.c.
Pirimiphos ethyl 20 % m/v e.c.

Commercially obtainable products:

"Librekto", a 5 % m/v cyhalothrin e.c.[1]
"Bayticol", a 2 % m/v flumethrin e.c.[2]
"Triatix", a 12,5 % m/v amitraz e.c.[3]
"Decatix", a 2,5 % m/v deltamethrin suspension concentrate.[3]

Tick species and strains

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

Experimental animals

Four male Friesland calves (8 months of age), of similar physical appearance and conformation, obtained from a single property.

Investigatory Shaw larval tests were undertaken using all but 1 of the compounds listed. Adult immersion tests were also carried out to assess the activity of 7 of the chemicals against engorged female ticks of the two strains. In addition, stall tests were used to evaluate the activity of amitraz, deltamethrin and flumethrin on calves infested artificially with the 'Braemar' or the 'Pot-the-Red' tick strains. Details of the treatments appear in Table 1.

TABLE 1 Ixodicides used in comparative tests

Ixodicide	Larval test	Adult immersion	Stall test
DDT	+	+	
Camphechlor	+		
Dioxathion	+		
Chlorfenvinphos	+		
Bromophos ethyl	+		
Pirimiphos ethyl	+		
Supamix*		+	
Amitraz	+	+	+
Cyhalothrin	+	+	
Cypermethrin	+	+	
Deltamethrin	+	+	+
Flumethrin	+	+	+

* Trade mark, Coopers Animal Health (Pty) Ltd. (A 50/50 composite of chlorfenvinphos and dioxathion)

Unfed larvae

The technique used was that described 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 conducting tests simultaneously.

A comparison was made of the susceptibility of larval offspring of the 2 strains 'Pot-the-Red' and 'Braemar' to the different chemicals. In addition, a comparison was made of the susceptibility of larvae of the 'Braemar' strain with that of larvae of some selected field strains. When chosing a strain for comparison, the strain least susceptible to the ixodicide under test was selected from 27 strains of *B. decoloratus* received from different areas of South Africa and tested at the Kwanyanga Research Station in recent years.

Adult immersion test

Adult immersion tests were carried out using the methods described by Coetzee *et al.* (1987) with the exception that only 20 ticks per group were utilized.

Stall tests

Stall tests were performed using the method described previously (Coetzee *et al.*, 1987).

One calf was infested with 3–4 week old larvae of the Kwanyanga susceptible reference strain 'Pot-the-Red'

[1] ICI South Africa (Pharmaceuticals) Ltd
[2] Bayer South Africa (Pty) Ltd
[3] Coopers Animal Health (Pty) Ltd

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and 3 calves were infested with the fenvalerate-resistant strain 'Braemar'. The animals were grouped and treated as shown in Table 2.

TABLE 2 Treatments

Calf number	Strain	Ixodicide	Target concentration % m/v
62	Pot-the-Red	Amitraz	0,025
60	Braemar	Amitraz	0,025
59	Braemar	Deltamethrin	0,005
61	Braemar	Flumethrin	0,005

RESULTS

Larval tests

The larval tests were conducted utilizing higher concentration ranges against the 'Braemar' strain than those used for 'Pot-the-Red'. LC 99 % values were, however, not obtained for the 'Braemar' strain with the use of DDT, camphechlor, chlorfenvinphos and the 4 pyrethroids: cyhalothrin, cypermethrin, deltamethrin and flumethrin. (It was unfortunately not possible to repeat these tests owing to limited resources.) In these instances statistical analyses of the data were therefore not undertaken, but the results are shown as a comparison against the susceptible 'Pot-the-Red' strain.

Organochlorines

Indications of a marked resistance were demonstrated by the 'Braemar' strain against both DDT and camphechlor.

Organophosphates

The resistance demonstrated by the 'Braemar' strain against dioxathion and pirimiphos ethyl was moderate, being of the order of 6-fold only. A comparison with 'Pot-the-Red' indicates that the differences in susceptibility to chlorfenvinphos could be due to the natural variations found in different tick strains. There was no difference in the susceptibility of either strain to bromophos ethyl. A comparison of the LC 99 % values for both strains with that of the selected field strain shows that both 'Pot-the-Red' and 'Braemar' are in fact more susceptible to dioxathion and bromophos ethyl. From the results obtained it is expected that the organophosphates used in this work could achieve satisfactory control of the 'Braemar' strain under normal conditions of field usage.

Diamidide

The 'Braemar' and 'Pot-the-Red' strains exhibited equal susceptibility to amitraz. 'Braemar' is in fact shown to be somewhat more susceptible to amitraz than the selected field strain.

Pyrethroids

Although no LC 50 % or 99 % values were obtained for the 'Braemar' strain, the maximum mortality in the larval tests, at concentrations of pyrethroid not far removed from the recommended field usage concentrations, indicate in all instances a high level of resistance. Compared with the results achieved against the 'Pot-the-Red' strain theoretical factors of resistance range from at least 250 to at least 4 000.

Adult immersion tests

In this test the susceptibility of 'Braemar' to 6 ixodicides was compared with that of 'Pot-the-Red'. A summary of the results appears in Table 4.

The results were submitted for statistical analyses although fewer than the ideal number of engorged female ticks were available for the immersion tests. Acceptable dose responses were obtained with DDT and deltamethrin. Percentage ER values were converted to the arcsin scale and multiple regression models were used to test for parallelism and colinearity between the strains. The relative potency (resistance) between 'Pot-the-Red' and 'Braemar' for DDT was 13 and for deltamethrin 24. The results obtained with the use of amitraz and supamix against both strains indicate susceptibility in relation to the recommended field usage concentrations of the commercial products containing these ixodicides.

Stall test

Following spraying with amitraz, the numbers of detaching females of both strains were greatly reduced by Day 4 and, apart from some minor fluctuations, never resumed a magnitude of any importance. The number of ticks ovipositing was much reduced in ticks collected on Day 1 and likewise never recovered during the rest of the trial.

In contrast, high numbers of ticks of the 'Braemar' strain continued to detach from those calves treated with either deltamethrin or flumethrin, apart from minor fluctuations. The numbers detaching from both calves were broadly similar.

Statistical analyses of the results (Table 5) using the chi-squared test show a significant difference between the 2 strains (P=0,162 or less) with regard to both oviposition and hatch for the pyrethroids used. The results using fenvalerate against 'Pot-the-Red' and 'Braemar' (Coetzee *et al.*, 1987) are included for comparative purposes.

The results achieved with the use of amitraz show no significant difference between the strains for oviposition but for hatch the difference is significant (P = 0,092) (Table 6). The percentage hatch is, however, low for both tick strains and amitraz should thus effect satisfactory control of the 'Braemar' strain under normal conditions of field usage.

Spraywash analysis

The amitraz spraywash was shown to contain 0,024 % m/v and the deltamethrin spraywash to contain 0,0047 % m/v.

DISCUSSION

With regard to the organophosphates used in this work, the 'Braemar' strain showed only a minor degree of resistance, which experience indicates would not be of practical importance in the field if regular treatment were to be practised.

The 'Braemar' strain proved to be fully susceptible to amitraz in both the larval and adult immersions tests, whilst virtually total inhibition of reproduction was achieved in the stall test.

The resistance demonstrated to all the pyrethroids used was striking in its severity. A dearth of resources prevented fuller investigations to determine LC 99 % values for all the ixodicides used in the larval test, but it is interesting to note that factors of resistance ranged from at least 250 (flumethrin) to at least 4 000 (cyhalothrin), suggesting that resistance is marked and widespread within this group of chemicals.

This is similarly shown by the adult immersion tests. It is emphasized with the use of flumethrin, which has marked adulticidal properties, where a maximum RI index of only 12 % was achieved with the highest concentration used on 'Braemar'.

Nolan, Roulston & Wharton (1977) have shown cross-resistance in *B. microplus* between DDT and permethrin and this has been confirmed at Kwanyanga with *B. decoloratus* (Baker & Jordaan, 1978 unpublished). It was, however, not possible to demonstrate in further work at the Kwanyanga Research Station, cross-resistance

TABLE 3 Results of larval test to determine the effects of various ixodicides on 2 strains of *B. decoloratus*

Ixodicides classified	Corrected (vs water control) maximum mortality (%) achieved		Concentration (%) of ixodicide required		Calculated LC 99 %*			FOR at LC 99 % level	
	PTR	B	PTR	B	FS	PTR	B	FS	Braemar
Organochlorines									
DDT	100	5	0,063	0,4	0,4	0,062	>10	ID	At least 161
Camphechlor	100	96	0,3	1,0	ID	0,1	2,2		22
Organophosphates									
Dioxathion	100	100	0,0025	0,01	0,015	0,001	0,0063	Nil	6
Chlorfenvinphos	100	63	0,0016	0,0025	ID	0,001	>0,0025		At least 2,5 (6)
Bromophos ethyl	99	100	0,01	0,01	0,019	0,01	0,0094	Nil	Nil
Pirimiphos ethyl	100	100	0,0025	0,04	ID	0,00038	0,0063		6
Diamidide									
Amitraz	100	100	0,001	0,00016	0,00096	0,0004	0,00013	Nil	Nil
Pyrethroids									
Cyhalothrin	100	17	0,0004	0,0025	ID	0,000025	0,1		At least 4 000
Cypermethrin	100	50	0,0004	0,0072	0,006	0,00016	0,1	ID	At least 600
Deltamethrin	100	34	0,0004	0,001	0,0004	0,00016	0,1	ID	At least 600
Flumethrin	100	7	0,0000063	0,001	ID	0,000004	0,001		At least 250

FS: Field Strain
PTR: Pot-the-Red
B: Braemar
(6): When calculated at LC 50 % level
*: Calculated by using line of best fit on logarithmic graph
ID: Insufficient data available

TABLE 4 Summary of adult immersion tests

Compound	Dose range	Pot-the-Red		Braemar	
		%ER range	ED50	%ER range	ED50
DDT	0,04 –10,0	17–100	0,12768	−2– 94	2,75675
Supamix	0,01 – 2,5	100–100	−0,01	84–100	−0,01
Amitraz	0,00063 – 0,16	99–100	−0,00063	99–100	−0,00063
Cyhalothrin	0,0016 – 0,4	95–100	−0,0016	15– 85	0,05035
Deltamethrin	0,0001 – 0,1	−13–100	0,00315	3– 73	0,05004
Flumethrin	0,000016– 0,004	75–100	−0,000016	−33– 18	−0,00025

ED50 = RI 50

TABLE 5 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
Amitraz	Pot-the-Red	74	100	92	294	29	18	<0,001	<0,001
Amitraz	Braemar	100	99	98	190	30	32	<0,001	<0,001
Deltamethrin	Braemar	100	99	99	978	79	92	<0,001	<0,021
Flumethrin	Braemar	66	98	98	896	89	93	0,025	0,162

TABLE 6 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
Amitraz	294	29	18	190	30	32	0,815	0,092

between DDT (FOR 7) and deltamethrin (Coetzee & Jordaan, 1984 unpublished). Therefore, although circumstantial evidence suggests that DDT cross-resistance is implicated in the resistance of the 'Braemar' strain to pyrethroids, this still requires confirmation.

The rapidity of development of resistance (apparently developed after 18 months of synthetic pyrethroid use in the field) casts serious doubts on the longevity of this group of compounds for tick control in southern Africa.

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