DETOXIFICATION AS A MECHANISM OF RESISTANCE IN A STRAIN OF THE CATTLE TICK *BOOPHILUS MICROPLUS* (CANESTRINI) RESISTANT TO ORGANOPHOSPHORUS AND CARBAMATE COMPOUNDS*

By W. J. Roulston,† C. A. Schuntner,† H. J. Schnitzerling,† and J. T. Wilson†

Strains of the cattle tick resistant to organophosphorus compounds were first found at Ridgelands in central Queensland in 1963 (Shaw and Malcolm 1964; Shaw 1966; Roulston, Stone, Wilson, and White 1968). Resistance of a different type was subsequently found at Biarra in south-eastern Queensland in 1966 (Roulston and Wharton 1967; Wharton 1967; Shaw, Cook, and Carson 1968). Ridgelands and Biarra strains of ticks both exhibit resistance to a wide range of organophosphorus and carbamate chemicals but differ in that resistance levels are higher and resistance extends to a wider range of chemicals in the Biarra strain. Biochemical investigations have shown that resistance in both strains is due to the presence of an acetylcholinesterase system which is relatively insensitive to inhibition by organophosphorus chemicals (Lee and Batham 1966; Roulston, Schnitzerling, and Schuntner 1968; Schuntner, Roulston, and Schnitzerling 1968).

In 1968 a third type of resistance to organophosphorus compounds was recognized at Mackay. The strain was provisionally classified as a Biarra type but further studies have shown that ticks of the Mackay strain differ from the Ridgelands and Biarra strains in their response to chemicals and in the biochemical basis of resistance.

Materials and Methods

In November 1967, following reports of control failure with coumaphos, engorged female ticks were obtained from a property at Constant Creek near Mackay by the Queensland Department of Primary Industries. Some of the larval progeny of these ticks were resistant to coumaphos (P. J. O'Sullivan, personal communication). The succeeding generation was derived from larvae surviving selection with coumaphos. Larvae of this generation were made available to the authors by Mr. P. J. O'Sullivan in March 1968. The strain, designated the Mackay strain, has since been cultured in this laboratory under selection by coumaphos.

Methods previously described were used to determine the resistance of larvae to various chemicals and the effectiveness of chemicals against parasitic ticks on stalled cattle (see Roulston and Wharton 1967; Roulston, Stone, Wilson, and White 1968); the level of acetylcholinesterase activity (see Schuntner, Roulston, and Schnitzerling 1968); the sensitivity of the acetylcholinesterase to inhibitors and the effectiveness of synergists (see Roulston, Schnitzerling, and Schuntner 1968), and the metabolism of coumaphos (see Roulston, Schuntner, and Schnitzerling 1966). The Mackay strain was compared with the Yeerongpilly (a susceptible reference strain), and the resistant Ridgelands and Biarra strains.

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† Division of Entomology, CSIRO, Long Pocket Laboratories, Private Bag No. 3, Indooroopilly, Qld. 4068.

Results and Discussion

The LC_{50} values of a number of chemicals* tested against larvae of the Yeerongpilly, Mackay, Ridgelands, and Biarra strains are shown in Table 1. Resistance factors calculated from these values and with values for the Yeerongpilly strain being used as a susceptible reference are also shown. The LC_{50} value for a chemical against a strain has been found to fluctuate and this has occurred particularly with the Yeerongpilly strain. Three of the resistance factors shown for the Mackay strain have been calculated on Yeerongpilly LC_{50} values which have differed from those already reported (Roulston 1969). This procedure has been adopted to preserve the relative resistance factors determined when the four strains were tested together on the one day.

TABLE 1

LC_{50} VALUES AND RESISTANCE FACTORS FOR A NUMBER OF CHEMICALS TESTED AGAINST MACKAY, YEERONGPILLY, RIDGELANDS, AND BIARRA TICKS

LC_{50} values given as percentage concentration of chemical in olive oil

Chemical	LC_{50} Values and Resistance Factors (in parenthesis)			
	Yeerongpilly	Mackay	Ridgelands*	Biarra*
Bromophos ethyl	0·28	0·39(2)‡	0·18(1)	0·78(3)
Carbamult	0·0012	0·014(12)	0·0082(7)	0·0070(6)
Carbaryl	0·0016	0·036(23)	0·021(13)	0·012(8)
Carbophenothion	0·41	3·6(9)	6·8(17)	16(39)
Chlorfenvinphos	0·026	0·40(15)	0·14(5)	0·31(12)
Ciodrin†	0·0075	0·054(7)	0·024(3)	0·15(20)
Coumaphos	0·048	0·14(9)§	0·079(2)	1·2(25)
Cyanox†	0·0076	0·027(4)	0·07(9)	1·68(221)
4,4′-DDT	0·71	1·0(2)‖	0·59(1)	0·68(1)
Diazinon	0·012	0·094(8)	0·12(10)	0·33(28)
Dimethoate	0·0019	0·12(63)	0·76(400)	0·19(100)
Dioxathion	0·075	1·8(24)	0·54(7)	0·99(13)
Dursban†	0·018	0·035(2)	0·034(2)	0·10(6)
Ethion	0·15	1·7(11)	0·41(3)	2·8(19)
Fenitrothion	0·029	0·039(1)	0·063(2)	2·5(86)
Fenthion	0·019	0·040(2)	0·047(3)	6·7(353)
Formothion†	0·0042	0·20(48)	0·80(190)	0·47(112)
Imidan†	0·018	0·14(8)	0·030(2)	0·036(2)
Malathion	0·41	0·60(1)	0·36(1)	0·66(2)

* Previously reported by Roulston (1969).

† Trade names.

‡ Calculated on a Yeerongpilly LC_{50} value of 0·23.

§ Calculated on a Yeerongpilly LC_{50} value of 0·016.

‖ Calculated on a Yeerongpilly LC_{50} value of 0·49.

The chemicals tested included those registered for tick control or regarded as potential acaricides. There were low factors of resistance in the Mackay strain to Dursban and bromophos ethyl, chemicals used to control Biarra ticks, but resistance to coumaphos and ethion, chemicals used to control Ridgelands ticks, was intermediate

* The chemical names have been given in Roulston, Stone, Wilson, and White (1968) except for Cyanox which is *O,O*-dimethyl-*O*-4-cyanophenyl phosphorothioate.

between the levels in the Ridgelands and Biarra strains. Resistance to two carbamates, carbaryl and carbamult, was higher in the Mackay strain than in either the Ridgelands or Biarra strains. The Mackay strain can be distinguished from the Ridgelands strain by its greater resistance to coumaphos and ethion, and from the Biarra strain by its much lower resistance to Cyanox.

The acetylcholinesterase activity and bimolecular rate constants (k values) for the reaction between inhibitors and acetylcholinesterase in larval homogenates of the four strains are shown in the following tabulation:

Strain	Acetylcholinesterase Activity (% of Yeerongpilly)	$10^{-4}k$ (litre mole^{-1} min^{-1})	
		Coroxon as Inhibitor	Diazoxon as Inhibitor
Yeerongpilly	100*	26	9·2
Ridgelands	14	8·7	0·43
Biarra	30	0·066	0·083
Mackay	27	22	6·9

* 100% = 440 μmoles acetylcholine hydrolysed/g/hr.

The acetylcholinesterase activity in the Mackay strain approximated to that in the Biarra, but the k values for the Mackay strain were close to those for the Yeerongpilly strain indicating that the sensitivity of the acetylcholinesterase to inhibition in the two strains was similar. These results suggested that the resistance mechanism in the Mackay strain differed from that in the Ridgelands and Biarra strains.

When ticks are treated with coumaphos some of the chemical is metabolized into the oxygen analogue or phosphate (coroxon), a much more potent inhibitor of acetylcholinesterase than the parent compound (Roulston, Schuntner, and Schnitzerling 1966). Thus, the toxic action of coumaphos largely depends on the amount of coroxon present. The metabolism of [^{32}P]coumaphos in Yeerongpilly, Biarra, and Mackay larvae was compared. The quantities of metabolites present 6 hr after dipping the larvae in 0·0003 and 0·00452% [^{32}P]coumaphos, which were approximately equi-toxic to Yeerongpilly and Mackay larvae, respectively, but non-toxic to Biarra larvae, are shown in Table 2.

At both dosages and particularly at the lower dosage there was far less coroxon present in Mackay larvae than in either Yeerongpilly or Biarra larvae. This could be the result of either a reduced potential for the formation of coroxon due to a decreased level of coumaphos, or by a greater hydrolysis of coroxon. At both dosages there were larger amounts of both of the major hydrolytic metabolites in Mackay larvae than in either Yeerongpilly or Biarra larvae. The larger amount of diethylthiophosphate could only result from an increased rate of hydrolysis of coumaphos and the larger amount of diethylphosphate can be attributed in all probability to an increased rate of hydrolysis of coroxon. The "minor metabolites" were also present in larger amounts in Mackay larvae. These results indicate that the smaller amount of coroxon found in Mackay larvae was due not to a slower rate of formation but rather to a greater rate of hydrolysis.

The amounts of coroxon in Yeerongpilly and Biarra larvae at both dosages were similar and in near proportion to the dosage. However, proportionately less diethylthiophosphate and diethylphosphate was produced in larvae of the three strains at the higher dosage than at the lower dosage, but this was not as marked in Mackay larvae as in larvae of the other two strains. These results indicate that there is a level of toxicant *in vivo* at which the rate of hydrolytic detoxification ceases to increase proportionately with the dose and that this dose is higher for Mackay larvae than either Yeerongpilly or Biarra larvae.

TABLE 2

METABOLISM OF [^{32}P]COUMAPHOS IN YEERONGPILLY, BIARRA, AND MACKAY LARVAE 6 HR AFTER TREATMENT

Strain	Concn. of Metabolites (as % of total internal radioactive materials)*				
	Coumaphos	Coroxon	Diethylthio-phosphate	Diethyl Phosphate	Minor Metabolites†
			0·0003% [^{32}P]coumaphos		
Yeerongpilly	26·9	21·8	11·3	37·8	2·2
Biarra	26·0	19·9	14·2	39·3	0·6
Mackay	4·3	0·7	27·4	54·3	13·3
			0·0045% [^{32}P]coumaphos		
Yeerongpilly	65·7	17·6	5·8	9·2	1·7
Biarra	62·0	18·2	6·8	11·1	1·9
Mackay	26·4	6·7	19·5	40·0	7·4

* Calculated from μg-equivalents of [^{32}P]coumaphos/g larvae.

† Probably desethyl derivatives of diethylthiophosphate and diethylphosphate.

The effectiveness of organophosphorus compounds against arthropods whose resistance is due to detoxification is generally enhanced by the addition of synergists (Metcalf 1967). However, the addition of tritolyl phosphate or piperonyl butoxide to coumaphos in the ratio of 5 : 1 failed to increase its effect against Mackay larvae.

Our results clearly show that the Mackay strain is distinct from the other strains resistant to organophosphorus compounds and that it has a degradative mechanism of resistance, one not previously encountered in *B. microplus*. The results of stall trials have indicated that satisfactory practical control might be expected from the use of either Dursban or a commercial mixture of bromophos ethyl and chlorfenvinphos.

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