

## The effect of acaricides on oviposition in a strain of *Boophilus decoloratus* (Koch) resistant to toxaphene and dioxathion

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### ABSTRACT

The effect of widely used acaricides on oviposition in a strain of *Boophilus decoloratus* (Koch) resistant to toxaphene and dioxathion was investigated. Engorged female ticks were dipped in acaricidal test solutions for 10 minutes within 24 hours after dropping from host animals. The acaricides used were toxaphene, dioxathion, chlorfenvinphos and amitraz; and were tested at the manufacturer's recommended concentration (field usage rates, FUR) and at 75 % of FUR. Both concentrations of chlorfenvinphos and amitraz had a strong inhibitory effect on oviposition (95 % inhibition) and hatchability of eggs. Toxaphene and dioxathion had less effect on oviposition (26 % inhibition) and egg hatchability. Most of the ticks exposed to toxaphene, dioxathion and amitraz were still alive after four weeks, while 30 % of those exposed to chlorfenvinphos died within the first week. These results suggest that the mode of action of amitraz was entirely on oviposition, probably by preventing the conversion of blood meal into eggs, or the release of eggs from the ovaries. The effect of chlorfenvinphos, on the other hand, was indirect as the ticks were killed before eggs could be laid. Most ticks were affected by this acaricide in the first week, although they were still alive.

### INTRODUCTION

Cattle ticks are a threat to cattle development throughout the world; they damage the hide and reduce the vitality, longevity and productivity of cattle both directly by their haematophagous activity and indirectly by transmitting fatal diseases (1). Traditionally, tick control has been effected by the use of contact ixodicides that affect either the tick itself, or its ability to reproduce. In Zambia, where one host and multi-host ticks parasitize cattle, the recommendation has been to execute control measures at regular weekly intervals. This has exerted an intense selection pressure on one-host ticks that stay on cattle for about 22 days leading to rapid development of resistance to acaricides (1).

Tick resistance to acaricides has been a recurring phenomenon throughout the world, and has usually appeared in succession to arsenic, hydrochlorinated and organophosphorous compounds (1). In Zambia, tick resistance to toxaphene and dioxathion has been reported previously (2, 3, 4). The measurement of resistance

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was based on increased survival of larvae exposed to these insecticides. The survival of adult female ticks of these resistant strains and their ability to oviposit successfully after exposure to acaricides were not investigated.

The objective of this study was to investigate the effect of widely used acaricides on oviposition in a strain of tick that is resistant to toxaphene and dioxathion.

## MATERIALS AND METHODS

### Tick Strain

The strain of *B. decoloratus* used in this study, was collected in 1984 from a Simmental herd, west of Lusaka. The herd was transferred from Mazabuka in 1981. No records of acaricide usage on these animals were available prior to 1981, but from 1981 to 1983, dioxathion was used in a dipping vat. The dip chemical was changed to dioxathion until control failures were experienced late in 1984.

A colony was established from engorged females. The ticks were indentified on arrival to the laboratory by the Taxonomy Section of the Livestock and Pest Research Centre. The colony was reared on steers and maintained in incubators at  $26 \pm 1^\circ\text{C}$  and  $80 \pm 5\%$  RH without further exposure to acaricides. The engorged females used in this study were from the third generation.

### Acaricides

The acaricides used and the field usage rate (FUR) and 75 % FUR concentrations tested were: Amitraz marketed as Triatix<sup>®</sup> by Coopers (Zambia) Limited, at 0.025 and 0.01875 % w/v; Chlorfenvinphos marketed as Supona Super<sup>®</sup> by Shell Chemicals (Zambia) Limited, at 0.05 and 0.0375 % w/v; Dioxathion marketed as Delnav DFF<sup>®</sup> by Coopers (Zambia) Limited, at 0.25 and 0.1875 % w/v; and Toxaphene marketed as Coppertox<sup>®</sup> by Coppers (Zambia) Limited, at 0.25 % and 0.1875 % w/v.

### Bioassay

The engorged ticks test method by Drummond *et al.* (5) was used. Engorged female ticks that detached normally from a steer no more than 24 hours earlier were used. Ticks were cleaned with water in a sieve, dried on absorbent paper and sorted into groups of 15. Each group was weighed and randomly allocated to a concentration. The average weight of ticks ranged between 220 and 260 mg. Groups were dipped in 100 mL plastic cups containing 50 mL of the test compound for 10 minutes after which the ticks were removed from the test solutions and placed on absorbent paper to dry.

Treated ticks were stuck, dorsal surface down, on a double sellotape attached to white formica boards. Ticks were attached in such a way that the front one quarter of their body protruded in front of the sellotape permitting unrestricted oviposition. The formica boards were then placed in incubators maintained at  $26 \pm 1^\circ\text{C}$  and  $80 \pm 5\%$  RH. Each treatment was replicated twice. Two groups of control ticks were dipped in water.

Oviposition was assessed weekly and eggs were collected using fine paint brushes and weighed at the same time. Percentage inhibition of oviposition was calculated using the formula of Mansingh and Rawlins (6). Tick mortality was

also recorded weekly and those showing movement of the appendages when prodded with a brush were classified as alive.

## RESULTS

The weights of eggs laid by the ticks over the six week period are shown in Figure 1. There was no significant difference ( $P > 0.05$ ) between the weight of eggs laid by females in the control groups and both concentrations of toxaphene and dioxathion. Ticks treated with chlorfenvinphos and amitraz laid fewer eggs than the control ticks, but the difference between the two compounds was not significant at both concentrations.

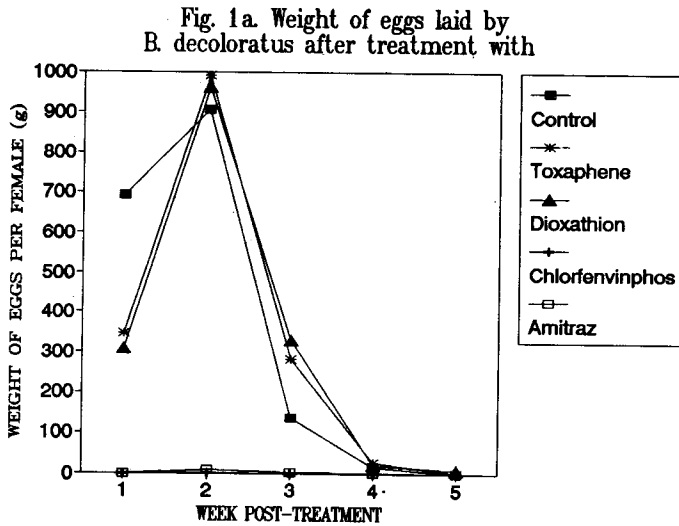


Figure 1 a. Average weight of eggs laid by control and acaricide-treated female *B. decoloratus* at FUR.

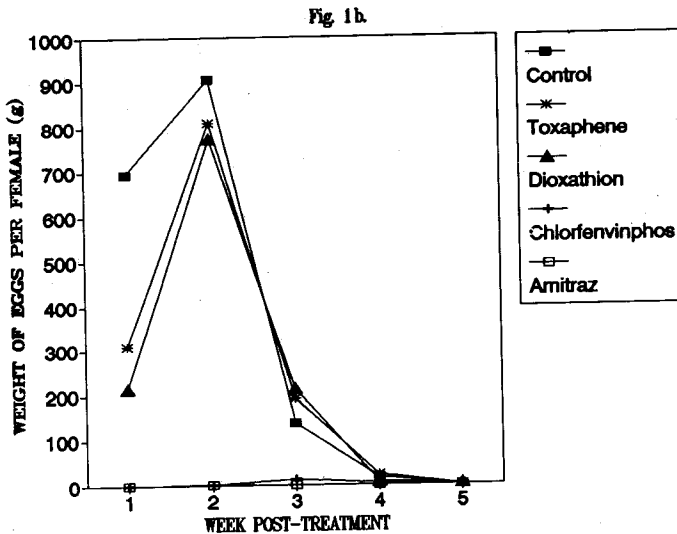
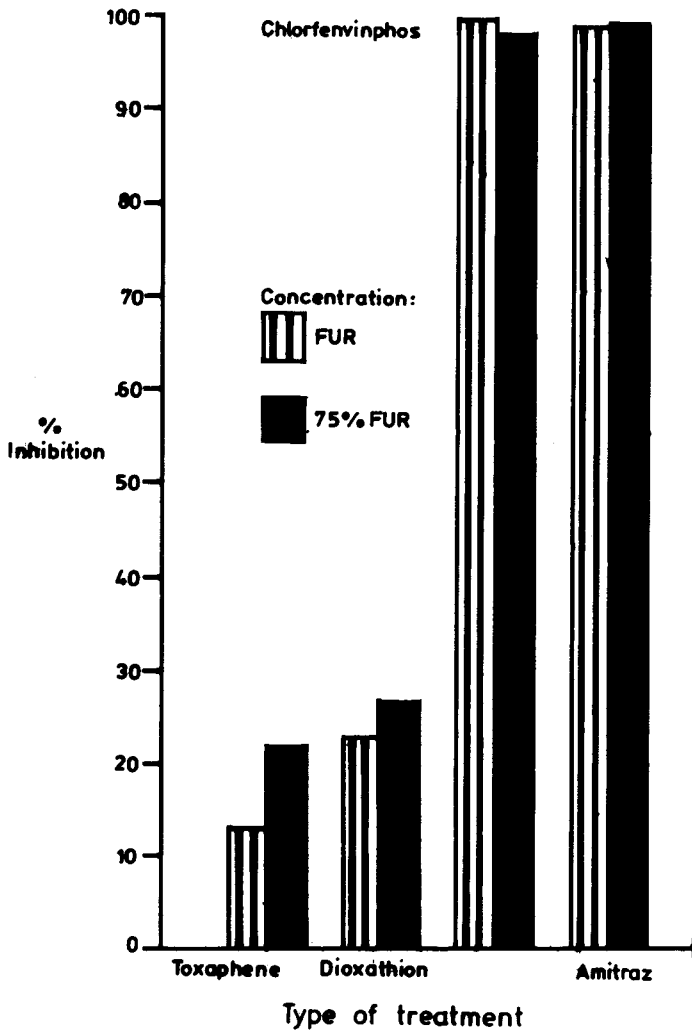


Figure 1 b. Average weight of eggs laid by control and acaricide-treated female *B. decoloratus* at 75 % FUR.

Both concentrations of chlorfenvinphos and amitraz inhibited oviposition by over 95 % while toxaphene and dioxathion were less effective, inhibiting oviposition by only 22 % and 24 % of the egg output in the control groups, respectively (Figure 2).



**Figure 2.** The Percentage inhibition of oviposition.

Oviposition by control ticks began on the fourth day after dropping off the host and continued for four weeks (Table I). In the fifth week, only 10 % of the ticks oviposited and no ticks oviposited in the sixth week. Some of the ticks treated with toxaphene and dioxathion oviposited four days after treatment, and 90-100 % of these ticks oviposited in the fifth week compared to 10 % in the control groups. Eggs from the control groups and both concentrations of toxaphene and dioxathion were viable. Some ticks treated with chlorfenvinphos and amitraz oviposited in the second week, and 0 - 10 % of these ticks oviposited eggs which were not viable.

**Table I**

*Percentage of B. decoloratus ovipositing at various times after acaricide treatment.*

Treatment % w/v	Week					
	1	2	3	4	5	6
Control	100	100	100	100	0	0
FUR Toxaphene	96.67	100	100	100	100	0
75% FUR	86.67	96.67	96.67	93.33	93.33	0
FUR Dioxathion	76.67	100	100	100	100	0
75% FUR	63.33	93.33	96.67	100	100	0
FUR Chlorfenvinphos	0	6.67	0	0	0	0
75% FUR	0	3.33	10.00	6.67	6.67	0
FUR Amitraz	0	10.00	6.67	3.33	0	0
75% FUR	0	3.33	0	0	0	0

Mortality results are given in Table II. Both concentrations of chlorfenvinphos produced over 30 % mortality in the first week and the figure reached 100 % by the sixth week. Mortality was observed in the fourth week for toxaphene, and in the fifth week for dioxathion and amitraz.

Table II

Percentage mortality of engorged adults of *B. decoloratus* after treatment with various acaricides.

Treatment % w/v	WEEK					
	1	2	3	4	5	6
FUR Toxaphene	0	0	0	0	20	74.07
75% FUR	0	0	0	6.67	20	62.96
FUR Dioxathion	0	0	0	0	3.33	51.85
75% FUR	0	0	0	0	13.33	48.15
FUR Chlorfenvinphos	33.33	43.33	43.33	43.33	76.67	100
75% FUR	36.67	46.67	53.33	56.67	70.00	100
FUR Amitraz	0	0	0	0	3.33	22.22
75% FUR	0	0	0	0	0	37.04

## DISCUSSION

The main objective of tick control is to reduce tick burdens on animals, tick populations on pastures and prevent tick-borne diseases. For *Boophilus* ticks, which are known to transmit diseases transovarially, it is important that oviposition is checked as an initial control strategy.

At FUR and 75 % of FUR, chlorfenvinphos and amitraz effectively inhibited oviposition, but dioxathion and toxaphene were ineffective against this strain. Inhibition of oviposition with chlorfenvinphos was accompanied by high mortality, indicating that its toxic action was by high initial kill of ticks and most ticks were clearly affected. The effect of amitraz was, conversely, mainly on oviposition as most ticks were still alive after the sixth week, but none laid viable eggs. This suggests that the females were still able to use the blood meal to maintain their own metabolism, but were unable to convert it into eggs. Alternatively, the oviposition process may have been inhibited after the formation of eggs. Other amidines have been shown to have potent inhibitory effect on oviposition in ticks and the hatchability of eggs (6, 7, 8, 9). Typically, the inhibitory effect of amidines on oviposition is followed by delayed death of the female ticks. Clenpyrin inhibits protein and probably lipid degradation whilst the degradation of carbohydrates is unaffected (9). Ticks treated with chlordimeform produce eggs, but do not oviposit; consequently the eggs are eventually resorbed by the ovaries (6). Mansingh and Rawlins (6) suggested that chlordimeform inhibits the release of eggs

from the ovaries and thus oviposition by interfering with muscular action. The mode of action of amitraz on our strain appeared to be similar to that of clenpyrin and chlordimeform. However, amitraz has different modes of action on different stages of ticks. It was extremely lethal to the larvae of our strain ( $LC_{50} > 400$  times lower than the FUR) (10), but did not kill engorged females.

Toxaphene and dioxathion did not significantly affect oviposition and egg hatchability, but altered the pattern of oviposition. Ticks treated with both concentrations of toxaphene and dioxathion laid about half the weight of eggs of the controls in the first week. In the second week, the quantities of eggs were similar, but subsequently treated groups laid more eggs than the control and the oviposition period was lengthened. Mansingh and Rawlins (9) found dioxathion as one of the most effective acaricides in inhibiting oviposition and egg hatchability in a susceptible strain of *B. microplus* (Can.), but was ineffective against our strain. The larvae of our strain were also resistant to toxaphene and dioxathion (10). These results suggest that during the development of resistance, acaricides lose their effect on processes that determine biotic potential in ticks. The effects of acaricides on tick survival, oviposition and egg hatchability are reduced, thus resulting in increased numbers of ticks on animals and subsequent control failures observed in the field.

Organophosphorus compounds act by inhibiting acetylcholinesterase in ticks (1). The difference between the effect of dioxathion and chlorfenvinphos could be due either to insensitive acetylcholinesterase (11), or differences in the rate of conversion to the more potent oxon analogue (12). The differences between the effect of dioxathion and chlorfenvinphos may be due to differences in their metabolism. Chlorfenvinphos is a triester phosphate and will inhibit acetylcholinesterase directly. Dioxathion, on the other hand, is a dithioester phosphate and has to be converted metabolically to the oxon analogue which inhibits acetylcholinesterase. The oxon metabolite is the more active toxicant, the parent thiono compound does not inhibit acetylcholinesterase (13). Furthermore, the bioactivation of dioxathion competes with detoxifying metabolic reaction such as hydrolysis, reduction, and glutathione-s-alkyl-transfer reactions. These competitive reactions could, therefore, reduce the potency of dioxathion as observed in this experiment. Chlorfenvinphos does not undergo bioactivation and would, therefore, be more effective against strains of ticks reported to be resistant to dioxathion (2, 3, 5). Further studies are required to verify the mechanisms responsible for resistance to dioxathion, but not to chlorfenvinphos before chlorfenvinphos could be unequivocally recommended for the control of strains of ticks resistant to dioxathion.

Slightly understrength concentrations (75 % FUR) were as effective as FUR and, in some cases, were more effective. We have no explanations for this disparity. This is not a recommendation for use of understrength dipwashes because they encourage the survival of resistant heterozygotes (14). However, it establishes the overkill incorporated into the FUR concentrations by manufacturers.

## CONCLUSION

The study shows that amitraz has a strong inhibitory effect on oviposition and hatchability of eggs in engorged females of a strain of *Boophilus decoloratus* resistant to toxaphene and dioxathion based acaricides. Chlorfenvinphos was, however, more effective in killing the ticks before they could lay eggs. Amitraz is,

therefore, recommended as the acaricide to use when resistance in ticks to organochlorine and organophosphorus is experienced on the farm.

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