Enhancement of permethrin efficacy in acaricide–attractant mixtures for control of the fowl tick *Argas persicus* (Acari: Argasidae)

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ABSTRACT

Three acaricides, permethrin, propoxur and diazinon, were tested against *Argas persicus* ticks in a test of susceptibility and in a multiple choice test in bioassay. A mixture of guanine hydrochloride and diatomaceous earth in saline was used as an attractant in bioassays, causing 53.1–95.7% assembly. The attractant was mixed with acaricides to reduce their repellency and enhance their efficiency in bioassays. Permethrin was the most toxic (LC_{95} at day $7 = 0.5$–1.4 mg m^{-2} depending on the developmental stage) and most repellent acaricide. The mortality of males in the bioassay was significantly higher (76.7–94.3%, $p < 0.01$) when acaricide in amounts of 16 and 160 μg of active ingredient per filter paper disc were mixed with attractant (0.5 mg per filter paper disc) instead of acaricide alone (20–45.7% mortality only). The mean permethrin residue on the tick body at the end of bioassay with the acaricide–attractant mixture was significantly higher (13.62 ± 11.64 ng) than in experiments without the attractant (less than 1 ng). Propoxur was less toxic (LC_{95} at day $7 = 0.9$–1.9 mg m^{-2}) and diazinon the least toxic (LC_{95} at day $7 = 2$–9.4 mg m^{-2}), both being not or only slightly repellent. Males and females also assembled on filter paper discs treated with propoxur without an attractant. Diazinon displayed significant mortality only in amounts of 0.1 and 1 mg of active ingredient per filter paper disc with or without the attractant. Therefore, the repellency of permethrin can be reduced and its effectiveness enhanced when used in a mixture with an attractant. No similar effect was observed with propoxur or diazinon.

Key words: Fowl tick, *Argas persicus*, acaricides, synthetic attractant, permethrin, diazinon, propoxur.

INTRODUCTION

Permethrin, a synthetic pyrethroid, has proved to be an effective repellent and toxicant for various blood-feeding arthropods, including hard and soft ticks (Schreck *et al*., 1978; Lane and Anderson, 1984; Mehr *et al*., 1986). Pressurized

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sprays of permethrin are commonly used for clothing and military uniform impregnation for personal protection against ticks (Schreck *et al*., 1980, 1982, 1986; Mount and Snoddy, 1983; Lane, 1989; Evans *et al*., 1990). A similar repellent effect on hard and soft ticks, including *Argas persicus* (Oken, 1818) and other *Argas* species, has also been observed in laboratory and field experiments with pyrethrum (Bar-Zeev and Gothilf, 1973, 1974; Kulkarni and Nair, 1985), which acts as a contact repellent. Repellency has also been mentioned for some other synthetic pyrethroids such as flumethrin (Gothe *et al*., 1984). Permethrin was found to be highly effective as a contact acaricide against the fowl tick *A. persicus* in our preliminary laboratory tests. Its repellent properties, however, limit its use for practical control of the fowl tick in hen-houses. Higher doses of permethrin are strongly repellent and discourage ticks from contact with treated areas. Moreover, acaricide doses close to the LC_{50} induce rather delayed toxification and survival of toxemic ticks for several weeks (syndrome of a slow death according to Uspenskiy (1982)). In laboratory experiments, Gothe *et al*. (1984) demonstrated that an assembly pheromone of *Argas walkerae* Kaiser and Hoogstraal, 1969 or its analogue, guanine, may attract ticks to filter paper discs impregnated with pyrethroid flumethrin and kill them.

Recently we successfully prepared an effective synthetic analogue of *A. persicus* assembly pheromone (Dusbábek *et al*., 1991). In the present report we tested its influence on the efficiency of permethrin and two other acaricides, propoxur and diazinon, on the fowl tick in a bioassay using acaricide–attractant mixtures.

MATERIALS AND METHODS

Ticks

Ticks of a field population of *Argas* (*Persicargas*) *persicus* (Oken, 1818), collected in hen-houses at Ipel'ský Sokolec, District of Levice, Slovakia during the spring periods of 1994–1996, were used. The ticks were held for several weeks to months before the experiments in the laboratory at $27 \pm 1^{\circ}C$ and $75 \pm 5\%$ relative humidity (RH) in darkness. The nymphs were allowed to feed on chickens before moulting. Unengorged nymphs 1–2 months after moulting and unengorged to semi-engorged males and females were tested.

Acaricides and attractant

Acaricides of three different chemical classes were used in the bioassays. Pyrethroid: permethrin, isomers *cis*:*trans* = 25:75 (Welcome Foundation Ltd, UK). Carbamate: propoxur of purity 96.7% (Bayer, Germany). Organophosphate:diazinon of purity 95.8% (Ciba-Geigy, Switzerland).

A simplified modification of a synthetic analogue of an assembly pheromone (Dusbábek *et al*., 1991) was used as follows: a mixture of 5 mg of guanine

hydrochloride and 5 mg of diatomaceous earth as pheromone carrier (1:1 w/w) was dispersed in 220 μl of 0.85% NaCl solution. The mixture was prepared 3 h before filter paper disc impregnation.

Tests of susceptibility to acaricides

Sheets of filter paper (10×10 cm) were each impregnated with 1 ml (at least 60 drops) of acaricide solutions in acetone and left to evaporate for 24 h at 23–25°C. They were then doubled up and closed at the margins by paper clips, forming test chambers of $10 \times 5 \times 0.7$ cm. The ticks were exposed inside the chambers for 24 h in a desiccator at 23–25°C and 100% RH. The ticks were then transferred to breeding vials with a 2×3 cm strip of filter paper and kept at 26°C and 100% RH. Mortalities were recorded daily for 21 days and that at the end of exposure of the ticks to the acaricide was considered to be day 1.

For each tick stage, five to six acaricide concentrations in the range of 0.1–0.000001% were used. Between ten and 20 ticks were used for each concentration and the LC_{50} and LC_{95} values were calculated from the tick mortality at days 1 and 7 using probit analysis.

Bioassay

The repellency and acaricide efficacy of each substance were tested in a bioassay. The multiple choice method in Petri dishes according to Leahy *et al*. (1973) was used. Groups of five nymphs of the same instar or five males or females were tested overnight (from 3.00 p.m. to 9.00 a.m.) in rough-bottomed Petri dishes (150 mm diameter) on eight filter paper discs (15 mm diameter). Only one filter paper disc was impregnated with the substance tested; the remaining seven stayed blank. Solutions of permethrin and propoxur in ethanol were used in amounts of 0.16–160 μg of active ingredient per filter paper disc, corresponding to an amount of 0.9–900 mg m^{-2}. An ethanol solution of diazinon was used in amounts of 0.001–1 mg of active ingredient per filter paper disc (=0.006–5.659 g m^{-2}). The attractant was used in an amount of approximately 0.5 mg per filter paper disc, both in the mixture with an acaricide or with pure ethanol (1:1 v/v) as the control. The position of the ticks in the Petri dishes was recorded at 9.00 a.m., i.e. after 18 h of exposure. Each experiment was replicated six to 12 times. The results of the assembly are presented in percentage degrees of preference (Otieno *et al*., 1985), calculated according to the following formula:

$$\left(1 - \frac{\text{Number of ticks assembled on control papers}/7}{\text{Number of ticks assembled on treated paper}}\right) \times 100$$

At the end of the bioassay, the ticks were transferred to the breeding vials and kept under breeding conditions. Their mortality was registered daily for 1 month after the bioassay. After this period, the ticks were allowed to feed on chickens and their further development was observed.

Detection of acaricide residue on the tick bodies

Ticks for residue detection were collected immediately at the end of the bioassay in a 0.2 ml methanol–acetone (1:1 v/v) mixture and transferred to Eppendorf vials. The permethrin and propoxur residues were ascertained by high-performance liquid chromatography (HPLC) on a Varian LC 5500 apparatus (Varian, Walnut Creek, CA, USA). For permethrin detection a Merck LiChrospher RP-100 column (125×4 mm, 5 μm) was used. Methanol (85%) was applied as a mobile phase (flow rate 1 ml min^{-1} at 225 nm, with 10 μl injections). In the case of propoxur identification, a Tessek Phenyl column (250×4 mm, 7 μm), with methanol (50%) as a mobile phase and a flow rate of 1 ml min^{-1} at 225 nm, was used.

Residues of diazinon were detected by gas chromatography with mass spectrometric detection (GC/MS) on a Hewlett Packard 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) directly coupled to a Kratos Profile mass spectrometer ITD 800 (Kratos, Manchester, GB). The tick samples were washed twice in 0.2 ml methanol–acetone (1:1 v/v), both washes were mixed and 50 ng of phenanthrene was added as the internal standard. The liquid was completely evaporated and the remaining residues then dissolved in 50 μl of a mixture of *t*-butylacetate-isoctane (2:8 v/v) and injected directly into the GC/MS instrument. The identification of diazinon was made by a comparison of observed spectra with a computerized spectral library.

RESULTS

Susceptibility to acaricides

The acaricide concentrations used in the test of susceptibility caused a tick mortality of 0–100%. The nymphal stages were generally more sensitive than the adult ticks. The toxicity of permethrin and propoxur was found to be higher than that of diazinon. The 40–60% mortality at day 7 of the males and females was caused by concentrations of 1.9–3.6 mg m^{-2} of diazinon, 0.3–0.4 mg m^{-2} of propoxur and 0.1–0.2 mg m^{-2} of permethrin. In the nymphs, these concentrations were 0.2–1.6 mg m^{-2} of diazinon, 0.2 mg m^{-2} of propoxur and 0.4 mg m^{-2} of permethrin. The mortality in these and higher concentrations increased gradually between days 1 and 7 and remained unchanged between days 8 and 21. Owing to this the LC_{50} and LC_{96} values for days 1 and 7 were different (Table 1). These differences were expressed as the factor of changes (LC_{50} or LC_{95} of day 1/LC_{50} or LC_{95} of day 7, respectively). These factors were highest in the diazinon and lowest in the propoxur treatment.

Repellency

The repellency of the acaricides depended strongly on the acaricide concentration and tick stage used. All the permethrin concentrations completely prevented the assembly of ticks on the acaricide–attractant mixture in the first

TABLE 1

LC_{50} and LC_{95} values for three acaricides at different developmental stages of *A. persicus* at days 1 and 7 after toxification

	LC_{50} ($mg\,m^{-2}$)			LC_{95} ($mg\,m^{-2}$)		
Acaricide, stage	Day 1	Day 7	Factor of change	Day 1	Day 7	Factor of change
Permethrin						
First stage nymphs	0.2	0.1	2.0	0.4	0.4	1.0
Males	0.7	0.2	3.5	4.8	1.4	3.4
Females	0.4	0.1	4.0	1.1	0.8	1.4
Propoxur						
Second stage nymphs	0.2	0.2	1.0	1.2	0.9	1.3
Males	1.0	0.3	3.3	3.8	1.5	2.5
Females	0.7	0.4	1.8	2.3	1.9	1.2
Diazinon						
First stage nymphs	0.5	0.2	2.5	28.0	2.0	14.0
Second stage nymphs	13.8	1.6	8.6	43.7	5.4	8.1
Males	13.2	3.6	3.6	60.0	11.0	5.4
Females	10.0	1.9	5.2	63.0	9.4	6.7

nymphal stage. The second stage nymphs tolerated concentrations of 0.16 and 1.6 μg of active ingredient per filter paper disc of permethrin solution in the attractant–acaricide mixture. The males and females tolerated a permethrin concentration of 160 μg of active ingredient per filter paper disc in the acaricide–attractant mixture and the males also assembled on filter paper discs treated with 0.16 μg of permethrin without an attractant (Table 2).

Only the highest propoxur concentrations (16 μg and 160 μg of active ingredient per filter paper disc) prevented assembly of the first nymphal stages on filter paper discs treated with the acaricide–attractant mixture. The assembly of second stage nymphs and adults was influenced only slightly by the presence of propoxur in the acaricide–attractant mixture. Propoxur alone was attractive to adults, mainly for females (Table 3).

The reactions of all the developmental stages to diazinon were similar and the repellency of this acaricide was expressed less than in permethrin. All the tick stages tolerated the weakest diazinon concentrations (0.001 and 0.01 mg of active ingredient per filter paper disc) and first stage nymphs and females even the concentration of 0.1 mg of active ingredient per filter paper disc and assembled on the synthetic attractant–acaricide mixture. Only an amount of 1 mg of diazinon per filter paper disc repelled the ticks and completely prevented assembly on the synthetic attractant. In addition, diazinon alone at the weakest concentration (0.001 mg of active ingredient per filter paper disc) attracted ticks and induced assembly on the filter paper disc (Table 4).

TABLE 2

Assembly of *A. persicus* on filter paper discs treated with synthetic attractant and permethrin

	First stage nymphs	Second stage nymphs	Males	Females
Synthetic attractant	91.2	95.5	95.7	53.1
Attractant–ethanol	87.5	91.7	81.2	66.7
Attractant–acaricide 0.16 μg	NS	90.5	NS	–
Attractant–acaricide 1.6 μg	NS	57.1	53.1	87.5
Attractant–acaricide 16 μg	NS	NS	75.3	NS
Attractant–acaricide 160 μg	NS	NS	68.8	85.7
Acaricide 0.16 μg	NS	NS	75.4	–
Acaricide 1.6 μg	NS	NS	NS	NS
Acaricide 16 μg	NS	NS	NS	NS
Acaricide 160 μg	NS	NS	NS	NS

The data are given as percentages of preference (see the Materials and methods section). Degrees less than 50 are considered to be insignificant at the level of 5%. (The number of positively responding ticks was less than the critical value of the binomial distribution: $10 < x_{crit} < 11$ at $n = 50$; see Likeš and Laga (1978)).

TABLE 3

Assembly of *A. persicus* on filter paper discs treated with synthetic attractant and propoxur

	First stage nymphs	Second stage nymphs	Males	Females
Synthetic attractant	91.2	95.4	95.7	53.1
Attractant–ethanol	85.7	88.3	90.5	66.7
Attractant–acaricide 0.16 μg	66.7	93.9	NS	NS
Attractant–acaricide 1.6 μg	57.1	57.1	73.5	78.6
Attractant–acaricide 16 μg	NS	NS	66.7	83.7
Attractant–acaricide 160 μg	NS	57.1	82.5	65.7
Acaricide 0.16 μg	NS	NS	NS	60.7
Acaricide 1.6 μg	NS	NS	NS	NS
Acaricide 16 μg	NS	NS	NS	78.6
Acaricide 160 μg	NS	NS	66.7	73.8

The data are given as percentages of preference (see the Materials and methods section). Degrees less than 50 are considered to be insignificant at the level of 5% (see Table 2 for an explanation).

Mortality in bioassay

The mortality of the ticks in the multiple choice bioassay experiments depended strongly on the tick developmental stage, the acaricide concentration used and the presence or absence of synthetic attractant (Tables 5 and 6). First stage nymphs appeared to be the most sensitive to a permethrin concentration of 1.6 μg of active ingredient per filter paper disc and showed mortalities of 50–60% when the acaricide was used either with or without attractant. The mortality of the second stage nymphs and adults, however, was significantly

TABLE 4

Assembly of *A. persicus* on filter paper discs treated with synthetic attractant and diazinon

	First stage nymphs	Second stage nymphs	Males	Females
Attractant	91.2	95.5	95.7	53.1
Attractant–ethanol	90.5	96.4	81.8	66.7
Attractant–acaricide 0.001 mg	90.4	87.5	89.1	–
Attractant–acaricide 0.01 mg	72.6	96.3	83.7	78.6
Attractant–acaricide 0.1 mg	71.4	NS	NS	66.7
Attractant–acaricide 1 mg	NS	NS	NS	NS
Acaricide 0.001 mg	68.8	51.3	71.4	–
Acaricide 0.01 mg	NS	NS	NS	NS
Acaricide 0.1 mg	NS	NS	NS	NS
Acaricide 1 mg	NS	NS	NS	NS

The data are given as percentages of preference (see the Materials and methods section). Degrees less than 50 are considered to be insignificant at the level of 5% (see Table 2 for an explanation).

TABLE 5

Percentage mortality of nymphal *A. persicus* in bioassay after feeding on chickens 30 days after acaricide toxification

	Acaricide		Attractant–Acaricide mixture	
	First stage nymphs	Second stage nymphs	First stage nymphs	Second stage nymphs
Permethrin				
0.16 μg	0.0	10.0	35.0	40.0**
1.6 μg	60.0	3.3	50.0	53.3**
16 μg	100.0	26.7	85.0	70.0**
160 μg	100.0	40.0	100.0	57.1
Control	5.7	1.3	5.7	1.3
Propoxur				
0.16 μg	0.0	0.0	0.0	0.0
1.6 μg	0.0	0.0	50.0**	0.0
16 μg	35.0	10.0	45.0	30.0
160 μg	45.0	30.0	65.0	30.0
Control	5.2	0.0	5.2	0.0
Diazinon				
0.001 mg	0.0	5.0	11.4*	5.0
0.01 mg	40.0*	35.0**	11.4	5.0
0.1 mg	94.3**	95.0	65.7	80.0
1 mg	100.0	100.0	97.1	100.0
Control	0.0	0.0	0.0	0.0

Mortality values marked by asterisks are significantly higher in the Student's *t*-test (*$p < 0.05$, **$p < 0.005$) than the corresponding values belonging to the same nymphal stage in neighbouring column.

TABLE 6

Percentage mortality of adult *A. persicus* in bioassay after feeding on chickens 30 days after acaricide toxification

	Acaricide		Attractant–Acaricide mixture	
	Males	Females	Males	Females
Permethrin				
0.16 μg	0.0	0.0	0.0	0.0
1.6 μg	20.0	20.0	13.3	13.3
16 μg	20.0	46.7	76.7**	86.7*
160 μg	45.7	86.7	94.3**	93.3
Control	0.0	0.0	0.0	0.0
Propoxur				
0.16 μg	0.0	6.7	0.0	6.7
1.6 μg	3.3	0.0	6.7	73.3**
16 μg	86.7	20.0	76.7	60.0*
160 μg	93.5	93.3	96.7	66.7
Control	0.0	0.0	0.0	0.0
Diazinon				
0.001 mg	0.0	25.0	0.0	0.0
0.01 mg	3.3	15.4	0.0	13.3
0.1 mg	100.0	100.0**	96.7	40.0
1 mg	100.0	100.0	100.0	100.0
Control	0.0	0.0	0.0	0.0

Mortality values marked by asterisks are significantly higher in the Student's *t*-test ($*p < 0.05$, $**p < 0.005$) than the corresponding values belonging to the same sex in the neighbouring column.

higher if permethrin was used with an attractant instead of acaricide alone. The curves of male mortality in the bioassays were quite different for acaricide alone and the acaricide–attractant mixture (Fig. 1).

The lowest propoxur concentration was completely ineffective for all developmental stages. A concentration of 1.6 μg of active ingredient per filter paper disc was partly effective only for the first stage nymphs and females in the acaricide–attractant mixture (Tables 5 and 6). The two highest concentrations (16 and 160 μg of active ingredient per filter paper disc) were more toxic to the adults than the nymphal ticks and there were no differences in the effectiveness of the pure acaricide and acaricide–attractant mixture. The male mortality curves were very similar (Fig. 2).

Diazinon in concentrations of 0.001 and 0.01 mg active ingredient per filter paper disc was ineffective or only slightly effective (Tables 5 and 6). Concentrations of 0.1 and 1 mg of active ingredient per filter paper disc were strongly toxic for all stages, causing 40–100% mortality both as pure acaricide as well as the acaricide–attractant mixture. No increase in mortality was observed using the acaricide–attractant mixture and the male mortality curves had very similar shapes (Fig. 3).

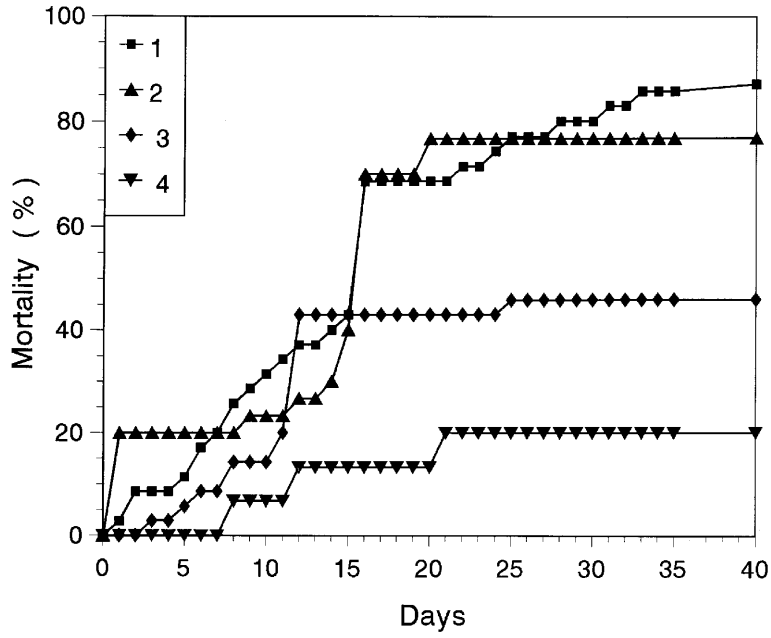

Fig. 1. Mortality curve of *A. persicus* males after toxification with permethrin in bioassay. (1) Attractant–permethrin mixture (16 μg of active ingredient per filter paper disc), (2) attractant–permethrin mixture (160 μg of active ingredient per filter paper disc), (3) permethrin (16 μg of active ingredient per filter paper disc) and (4) permethrin (160 μg of active ingredient per filter paper disc).

Acaricide residues on the tick bodies

The positive role of the attractant in the acaricide–attractant mixture for male *A. persicus* toxification with permethrin was confirmed by the detection of permethrin residues on the bodies of the ticks. Significantly more ($p < 0.01$ in the Student's *t*-test) permethrin was detected on the tick bodies at the end of the bioassay with the acaricide–attractant mixture than with the acaricide alone, when a concentration of 160 μg of active ingredient per filter paper disc was used. With diazinon and propoxur this positive effect of the attractant was not confirmed (Table 7). Relatively high diazinon residues were detected in the male ticks, both in the pure acaricide and acaricide–attractant mixture with no significant differences between them ($p > 0.05$). Only traces of propoxur or none at all were detected on the male tick bodies.

DISCUSSION

Permethrin and propoxur proved to be more toxic for the fowl tick, *A. persicus*, than diazinon. The LC_{50} and LC_{95} values of diazinon were approximately five to

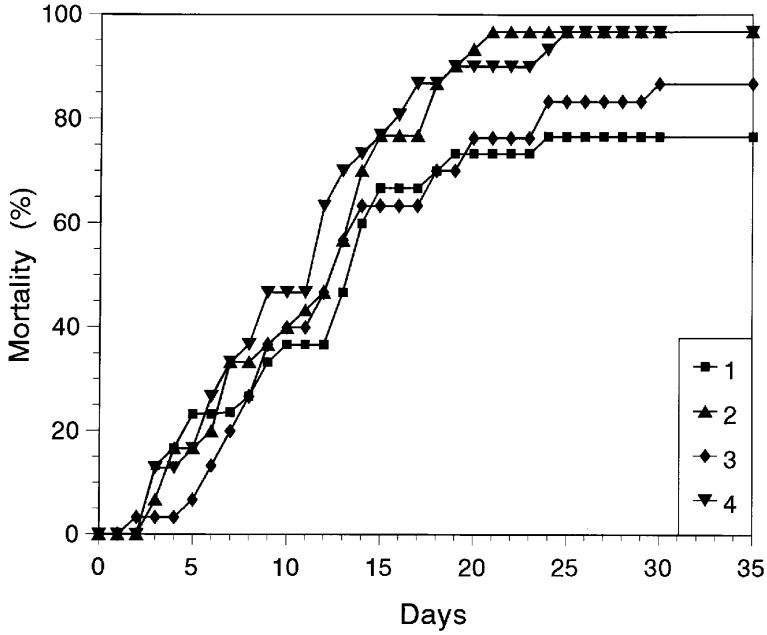

Fig. 2. Mortality curve of *A. persicus* males after toxification with propoxur in bioassay. (1) Attractant–propoxur mixture (16 μg of active ingredient per filter paper disc), (2) attractant–propoxur mixture (160 μg of active ingredient per filter paper disc), (3)-propoxur (16 μg of active ingredient per filter paper disc) and (4) propoxur (160 μg of active ingredient per filter paper disc).

ten times higher than in the other two acaricides. The mortality of the ticks increased slowly in all cases up to day 7 in the test of susceptibility to the acaricides and up to day 30 in the bioassay experiments. The effective acaricide doses in the bioassays were ten or more times higher than the LC_{50} or LC_{95} values calculated for day 1 or day 7. The syndrome of a slow death (Uspenskiy, 1982) was recorded in all cases.

Guanine hydrochloride mixed with diatomaceous earth in saline proved to be an appropriate attractant for the fowl tick, being sufficiently stable (Dusbábek *et al*., 1997) and able to reduce the repellent properties of permethrin. In bioassay, the adult ticks were able to assemble on the attractant mixed with toxic acaricide concentrations. A higher toxification of the ticks has also been confirmed by the identification of a significantly higher amount of permethrin residues on the tick bodies in the bioassays with the permethrin–acaricide mixture with an amount of 160 μg of active ingredient per filter paper disc.

The repellency tests with diazinon indicated a weak repellency of this acaricide which was prevented completely in both of the weakest concentrations in the acaricide–attractant mixture. Propoxur displayed a weak repellency for first stage nymphs, but not for the other developmental stages.

In some cases assembly of the ticks also occurred after exposure to the

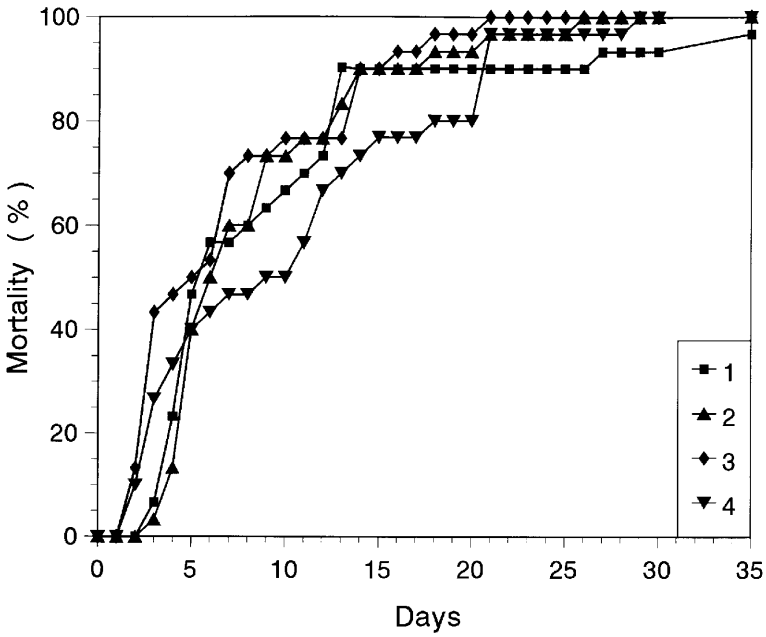

Fig. 3. Mortality curve of *A. persicus* males after toxification with diazinon in bioassay. (1) Attractant–diazinon mixture (0.1 mg of active ingredient per filter paper disc), (2) attractant–diazinon mixture (1 mg of active ingredient per filter paper disc), (3) diazinon (0.1 mg of active ingredient per filter paper disc) and (4) diazinon (1 mg of active ingredient per filter paper disc).

TABLE 7

Acaricide residues (ng) on the bodies of male *A. persicus* at the end of the bioassays with the highest concentrations of acaricide and the acaricide–attractant mixtures (mean of ten samples ± SD)

	Acaricide	Acaricide–Attractant mixture
Permethrin (160 μg)	Traces (<1)	13.62 ± 11.64
Propoxur (160 μg)	0.0	Traces (<1)
Diazinon (1 mg)	404.21 ± 313.68	317.61 ± 311.96

weakest concentrations of acaricides without attractant. With the exception of propoxur, these acaricide concentrations were not toxic in bioassay and were probably not registered by the tick gustatory organs. The assembly could be induced in these cases by physical changes on treated filter paper discs, perceived by the mechanoreceptoric system of the argasid palpal organs and by the presence of NaCl crystals from saline which support assembly (see Dusbábek *et al*., 1991). However, the assembly of females and males on high

concentrations of propoxur indicates the direct attractiveness of this acaricide for adult ticks.

Our results correspond with the results obtained by Gothe *et al*. (1984) who tested the influence of flumethrin on the assembly of *Argas* (*Persicargas*) *walkerae*, using the natural pheromone and its analogue, guanine. As in our experiments, the assembly of *A. walkerae* on tested filter paper discs decreased with an increased flumethrin concentration. First stage nymphs were also the most sensitive to toxification and their assembly on guanine as a pheromone analogue in the bioassay depended on the guanine concentration. However, the mortality of *A. walkerae* increased with an increase in the flumethrin concentration up to the optimal concentration and decreased when this optimum was surpassed, probably owing to the increased repellency effect of flumethrin. Therefore, the prevention of an acaricide repellent effect at higher concentrations by the pheromone analogue (as in our experiments with male *A. persicus*) was not observed by these authors.

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