ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2013, Vol 20, No 3, 447–451
www.aaem.pl

Evaluation of the effect of various concentrations of selected pyrethroids on the development of *Dermacentor reticulatus* eggs and larvae

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Buczek A, Bartosik K, Kuczyński P. Evaluation of the effect of various concentrations of selected pyrethroids on the development of *Dermacentor reticulatus* eggs and larvae. Ann Agric Environ Med. 2013; 20(3): 447–451.

Abstract

The Palearctic three-host species *Dermacentor reticulatus* contributes to the circulation of numerous pathogens in the environment. Reduction of its abundance may therefore decrease the risk of tick-borne diseases in a given area. The aim of the study is to determine the effect of various concentrations of three pyrethroids – deltamethrin (D), cypermethrin (C), and alpha-cypermethrin (AC) on the development of *D. reticulatus* eggs and larvae. 217 engorged *D. reticulatus* females were examined in the investigations. After the feeding period, they were sprayed with 0.015625%, 0.03125%, 0.0625%, and 0.125% solutions of D, C, and AC, and kept at a temperature of 25°C and 90% relative humidity throughout the preoviposition and oviposition periods. Eggs laid by females were kept in the same conditions until larval hatch. Based on the results obtained, parameters of the course of maturation and oviposition, as well as parameters of embryonic development, were determined. The investigations showed that the pyrethroids tested prolonged the egg maturation period, reduced the number and weight of eggs, and caused disturbances in embryogenesis in *D. reticulatus*. Upon treatment with as little as 0.015625% AC, larvae did not develop and all eggs died 1–2 days after oviposition. C led to a decreased percentage of normal larval hatch – 31.96%, 15.51% and 12.5%, respectively, after using three increasing concentrations (control 98.15%), and a high rate of egg and embryo mortality. The least detrimental effect on the *D. reticulatus* maturation and embryonic periods was exerted by deltamethrin (82.74%, 84.37% and 16.80% of normal larvae in treatment with the three concentrations). Morphological anomalies were observed in larvae during the experimental period. AC appeared to have the most toxic effect during the maturity and egg development periods, while C exhibited lower toxicity. Application of these pyrethroids in engorged *D. reticulatus* females exerts distant effects that lead to substantial reduction of tick offspring abundance.

Key words

Dermacentor reticulatus, tick control, deltamethrin, cypermethrin, alpha-cypermethrin, anomalies

INTRODUCTION

The tick *Dermacentor reticulatus* occurs in meadow and river valley ecosystems in the Palearctic. In recent years, its distribution range in Europe has expanded and currently it encompasses suburban areas and city peripheries. The biological features of *D. reticulatus*, particularly its three-host developmental cycle, wide host range and high fertility, as well as transmission of numerous pathogens [1, 2, 3, 4], ensure a significant role of the species in the maintenance of tick-borne disease foci in a given area. Therefore, reduction of *D. reticulatus* population abundance is fundamental for the protection of human and animal health. Investigations into the application of chemical compounds in tick control are primarily focused on their insecticidal activity towards adult forms. In contrast, data on their effect on the early developmental stages are sparse, although control of the abundance of three-host ticks at these developmental stages could efficiently reduce the risk of interspecies pathogen transmission during feeding on various hosts. The aim of the presented study was to examine for the first time the effect of pyrethroid application during the development of *D. reticulatus* eggs and larvae.

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Received: 29 December 2012; accepted: 19 March 2013

MATERIAL AND METHODS

Adult *D. reticulatus* were collected by the flagging method during their spring activity in 2003 in the surroundings of Lublin (51°15'N, eastern Poland) [5]. Prior to the experiments, the ticks were kept under laboratory conditions at a temperature of 4°C and 90% relative humidity (RH). Methods devised by one of the authors (A. Buczek) were employed in the study. Before being placed on the shaved skin of albino New Zealand rabbits (*Oryctolagus cuniculus*), unengorged *D. reticulatus* females were weighed using a RADWAG XA WPA 120/C/1 digital analytical laboratory balance with an accuracy of 0.01 mg. To ensure the proper course of feeding, 15 females and 5 *D. reticulatus* males were placed on each of the rabbits. The experiments were carried out at room temperature and ca. 50% RH. The ticks were checked daily, at the same time, and when detached, engorged specimens were weighed and transferred onto filter paper discs in rearing chambers. One engorged female was placed in one rearing chamber and sprayed with 20 µl of a given pyrethroid solution using a 0.2–50 µl micropipette with an accuracy of 0.5–2% (O.G. Labsystems, Helsinki, Finland).

167 females were treated with pyrethroids. Simultaneously, a control group composed of 50 females was created, in which the same procedures as in the experimental acaricide application were employed, but the females were not sprayed with the tested chemical compounds. All females were kept in the dark at the temperature of 25°C and 90% RH until the end of oviposition. In total, 217 *D. reticulatus* female were used in the experiments.

The experiment was checked every day in order to trace the course of egg laying by females affected by the different pyrethroid concentrations and females in the control group. After the end of the oviposition period, the females and eggs were weighed. Respective egg batches were left in thermostat chambers under the aforementioned conditions throughout the embryonic development period until the end of larval hatch. Next, the number of dead eggs and embryos, as well as the number of larvae with developmental anomalies, larvae with hatching disturbances, and normal larvae, were examined under a stereoscopic microscope. In the case of embryos, the embryogenesis stage in which growth inhibition occurred was determined. In the first stage, the eggs retained their round shape and no divisions of the embryonic material were visible through the egg shell; in the subsequent stage, changes in the structure of embryos resulting from cell divisions, and in the third stage, leg buds and elements of the gnathosoma were visible through the egg shell. Based on the results obtained, parameters of the maturation and oviposition processes and embryonic development parameters were assessed.

Parameters of the eggs maturation and oviposition course. Egg laying frequency (ELF) – the number of females capable of laying eggs per number of engorged females in the respective group.

Female postoviposition weight (FPW) – weight of female body after oviposition (in grams). Female oviposition weight loss (FOWL) – an index of the percentage of weight loss during oviposition calculated as the ratio of the difference between engorged female weight and postoviposition weight/engorged female weight (values expressed in %). Egg mass weight (EMW) – the total weight of eggs laid by a female (in mg). Egg conversion factor (ECF) represents engorged female weight used for egg production. Egg amount (EA) – the total number of eggs laid by a female.

Parameters of embryonic development. Hatching frequency (HF) – percentage of egg batches in the tested group with at least one hatched larva (in %).

Embryogenesis period (EP) –period between the beginning of oviposition to hatching of the first larva (in days). Hatching success (HS) determines the proportion of laid eggs, from which larvae hatched (in %).

Tested acaricides. The experimental compounds included K-Othrine 2.5 flow (Roussel Uclaf, France) containing deltamethrin, Kordon 10 WP (AgrEvo Environmental Health Ltd., UK) containing cypermethrin, and Alfasect 5SC (ASPRAN s.c. Jaworzno, Poland) containing alpha-cypermethrin. The activity of 0.015625%, 0.03125%, 0.0625%, and 0.125% solutions of deltamethrin, cypermethrin, and alpha-cypermethrin were tested. The amount of the active agent per single dose (20µl) of the acaricide applied is presented in Table 1.

Table 1. Quantity of active substance in 20 µl of a given pyrethroid solutions applied as a single dose (in µg)

Concentration of the solution (%)	Content of active substance in 20 µl of solution (in µg)		
	Deltamethrin	Cypermethrin	Alpha-cypermethrin
0.01562	0.07812	0.3125	0.1562
0.03125	0.1562	0.625	0.3125
0.0625	0.3125	1.25	0.625
0.125	0.625	2.5	1.25

Statistical analysis. The results were analyzed using statistical package STATISTICA 5 and Microsoft Excel XP. Analysis of the differences in the distribution of the results depending on the tick group tested was performed using the Mann-Whitney U test and Kruskal-Wallis H test. The test probability at $p<0.05$ was assumed significant and at $p<0.01$ highly significant.

RESULTS

The use of the pyrethroids tested led to a reduction of the percentage of egg-laying *D. reticulatus* females, together with the increasing concentrations of the chemical substance. 0.125% of deltamethrin, cypermethrin, and alpha-cypermethrin, was the largest concentration of the pyrethroid solutions at which oviposition took place. Under their effect, 50.0%, 50.0%, and 16.7% females, respectively, were able to lay eggs (control – 100%) (Fig. 1).

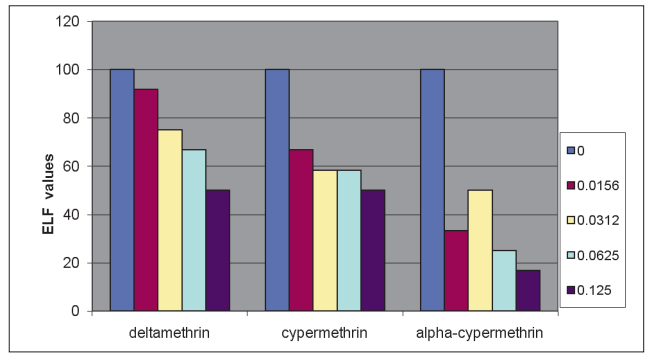

Figure 1. Egg laying frequency (ELF) in *Dermacentor reticulatus* females under the influence of different concentration of deltamethrin, cypermethrin and alpha-cypermethrin.

The preoviposition period was statistically significantly prolonged (Tab. 2) as the concentration of the pyrethroids applied increased. At deltamethrin concentrations of 0.015625-0.125%, the egg maturation period lasted from 7.636±2.336 – 13.333±3.830 days; in the case of cypermethrin and alpha-cypermethrin it was 10.0±3.295 – 15.500±4.183 days and 12.750±4.573 – 17.50±10.607 days, respectively (2.167±0.648 days in the control). During the oviposition period, the oviposition weight loss in *D. reticulatus* females treated with pyrethroids was lower than in the control group (Tab. 2).

The mean egg mass weight (EMW) and the egg conversion factor (ECF) exhibited by eggs laid by females treated with deltamethrin, cypermethrin, and alpha-cypermethrin, decreased together with the increasing concentrations of the solutions and were statistically significantly lower than those in the control (Tab. 2). Similarly, the mean egg amount

Table 2. Parameters of eggs maturation and oviposition course in *Dermacentor reticulatus* females under the influence of different concentration of deltamethrin, cypermethrin and alpha-cypermethrin at temp. 25°C and 90% RH

Chemical	Concentration (%)	Preoviposition (days)			FPW[1] (g)			FOWL[2] (%)			EMW[3] (mg)			ECF[4]		
		M	SD	p	M	SD	p	M	SD	p	M	SD	p	M	SD	p
Deltamethrin N=64	0.01562	7.636	2.336	0.0013	0.174	0.037	0.0004	54.562	13.570	0.0005	0.140	0.050	0.0005	0.353	0.102	0.0002
	0.03125	8.333	3.354	0.0001	0.159	0.032	0.0084	61.453	8.402	0.0102	0.151	0.052	0.0037	0.362	0.122	0.0024
	0.0625	9.375	1.685	0.0048	0.183	0.051	0.0059	56.575	12.036	0.0036	0.099	0.031	0.0000	0.234	0.073	0.0000
	0.125	13.333	3.830	0.0195	0.243	0.040	0.0004	45.971	12.580	0.0005	0.093	0.018	0.0004	0.206	0.049	0.0004
Cypermethrin N=58	0.01562	10.000	3.295	0.0000	0.198	0.054	0.0019	49.952	10.101	0.0001	0.099	0.018	0.0000	0.258	0.069	0.0000
	0.03125	11.000	3.109	0.0000	0.155	0.068	0.5092	61.655	15.191	0.1868	0.109	0.061	0.0029	0.278	0.167	0.0054
	0.0625	11.857	3.485	0.0000	0.197	0.019	0.0013	55.759	4.076	0.0027	0.090	0.059	0.0023	0.195	0.107	0.0013
	0.125	15.500	4.183	0.0001	0.245	0.013	0.0048	29.072	1.735	0.0048	0.051	0.030	0.0048	0.149	0.089	0.0048
Alpha-cypermethrin N=45	0.01562	12.750	4.573	0.0013	0.213	0.002	0.0048	36.170	9.822	0.0048	0.084	0.004	0.0048	0.253	0.046	0.0048
	0.03125	13.167	2.317	0.0001	0.219	0.084	0.0284	47.907	15.849	0.0102	0.070	0.030	0.0013	0.176	0.082	0.0013
	0.0625	13.000	2.646	0.0048	x	x	x	x	x	x	x	x	x	x	x	x
	0.125	17.500	10.607	0.0195	x	x	x	x	x	x	x	x	x	x	x	x
Control N=50		2.167	0.648	-	0.127	0.023	-	69.547	5.801	-	0.221	0.044	-	0.531	0.119	-

[1] female postoviposition weight – weight of female body after oviposition
[2] female oviposition weight loss – an index of the percentage of weight loss during oviposition calculated as the ratio of the difference between engorged female weight and postoviposition weight/engorged female weight
[3] egg mass weight – total weight of eggs laid by a female
[4] egg conversion factor – engorged female weight used for egg production
N – number of ticks used in the experiment

in a single oviposition batch decreased after the females had been sprayed with the tested pyrethroids, compared with the control (Fig. 2). Females treated with 0.015625% alpha-cypermethrin laid the lowest mean number of eggs (512) (3966.3 eggs in the control). Larval hatching frequency differed upon using the different concentrations of the tested pyrethroids (Tab. 3). Even the lowest concentrations of cypermethrin and alpha-cypermethrin led to a decrease in the number of egg batches from which *D. reticulatus* larvae hatched. In the case of deltamethrin, the hatching frequency declined only after application of concentrations of 0.0625 and 0.125%.

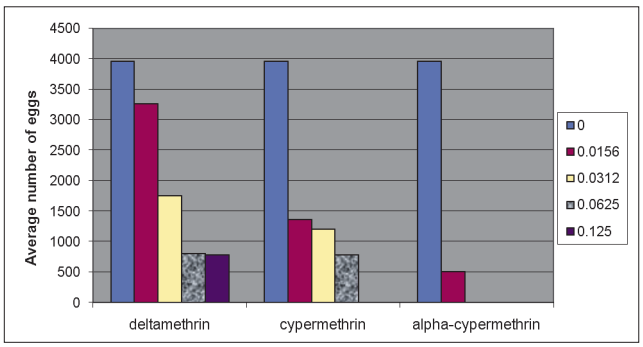

Figure 2. Egg amount (EA) in *Dermacentor reticulatus* females under the influence of different concentration of deltamethrin, cypermethrin and alpha-cypermethrin.

Compared to the control, the embryonic development in *D. reticulatus* was prolonged in a statistically significant manner after application of all the pyrethroid concentrations (Tab. 2). Additionally, statistically significant differences in the embryogenesis length were found upon application of the different concentrations of the tested compounds. The rate of normal larval hatching ranged from 82.74 – 88.57% in the experiments carried out with the use of deltamethrin in the concentration of 0.015625 – 0.0625% and significantly

Table 3. Average values of parameters of embryonic development in *Dermacentor reticulatus* under the influence of different concentration of deltamethrin, cypermethrin and alpha-cypermethrin at temp. 25°C and 90% RH.

Chemical	Concentration (%)	HF[1] (%)	EP[2] (days)	HS[3] (%)
Deltamethrin N=64	0.01562	100	22.36	82.76
	0.03125	100	22.22	84.37
	0.0625	87.5	23.146	89.07
	0.125	83.3	21.80	16.87
Cypermethrin N=58	0.01562	87.5	28.71	31.96
	0.03125	71.4	30.60	15.51
	0.0625	57.1	29.00	12.50
	0.125	50	25.33	-
Alpha-cypermethrin N=45	0.01562	75.0	27.33	0.00
	0.03125	66.7	29,00	-
	0.0625	0	-	-
	0.125	0	-	-
Control N=50		100	12.20	98.15

[1]HF – hatching frequency, percentage of egg batches in the tested group with at least one hatched larva
[2]EP – embryogenesis period: period between the beginning of oviposition to hatching of the first larva
HS – hatching success determines the proportion of laid eggs, from which larvae hatched

decreased (16.80%) upon application of its 0.125% solution (control 98.15%). Together with the increasing concentrations of this compound, egg mortality rates increased. The embryos usually died in developmental stage I or II. In addition, larval specimens with morphological deformities were found which included leg oligomely (loss), atrophy (reduction), and symely (change of position).

A majority of the *D. reticulatus* eggs observed upon application of cypermethrin died directly after oviposition or in the first developmental stage. The number of normal

Table 4. Course of embryonic development in *Dermacentor reticulatus* under the influence of deltamethrin, cypermethrin and alpha-cypermethrin.

PARAMETER	Deltamethrin concentration (%)				Cypermethrin concentration (%)			Alfa-cypermethrin concentration (%)	Control
	0.01562	**0.03125**	**0.0625**	**0.125**	**0.01562**	**0.03125**	**0.0625**	**0.01562**	
Normal larvae (%)	82.74	84.37	88.57	16.80	31.96	15.51	12.50	0.00	98.15
Larvae with developmental anomalies (%)	0.02	0.00	0.50	0.07	0.00	0.00	0.00	0.00	0.00
Larvae with hatching disturbances (%)	1.30	1.28	1.12	1.00	0.29	1.08	3.74	0.00	0.13
Dead eggs (%)	2.00	3.37	5.34	59.20	66.86	8.13	8.89	100	0.07
Inhibition in embryogenesis (%)	13.95	10.98	4.47	22.94	0.88	75.29	74.87	0.00	1.65
I stage (%)	36.34	56.14	52.78	51.96	100	83.15	91.57	0.00	30.06
II stage (%)	42.91	40.06	33.33	47.01	0.00	15.64	8.43	0.00	57.48
III stage (%)	20.74	3.80	13.89	1.03	0.00	1.21	0.00	0.00	12.47

larvae decreased and ranged from 31.96 – 12.50% along with the increasing concentrations of the tested substance (Tab. 4). The use of alpha-cypermethrin resulted in 100% mortality of eggs laid by females.

DISCUSSION

Although pyrethroids are widely used for tick control in field conditions [6, 7, 8], little is known about their effect on the non-parasitic phase of the developmental cycle of these arthropods, particularly in the early developmental stages of various species. The presented study shows that exposure of *D. reticulatus* females to deltamethrin, cypermethrin, and alpha-cypermethrin exerts distant effects, reflected by disturbances in the course of egg maturation, oviposition and embryonic development. In consequence, the female reproductive performance and the number of viable larvae declined, while egg and embryo mortality increased in the stage of cleavage and organogenesis.

Among the tested compounds, alpha-cypermethrin and cypermethrin exerted the most detrimental effect on ticks in the non-parasitic phase of the developmental cycle. Compared with the other pyrethroid tested, deltamethrin had lower toxic effects. The longest preoviposition period (15.50±4.183 days) was observed after application of 0.125% cypermethrin, and even at the lowest concentrations of this substance, egg maturation was nearly five-fold longer than in the control, in which it proceeded for 2.167±0.648 days.

The increased concentration of the active agent increased the length of the preoviposition period. The length of this period is specific for various tick species and depends primarily on ambient temperature. The chemical substances applied lowered the effectiveness of egg maturity, manifested by changes in egg laying frequency exhibited by females treated with pyrethroids. Alpha-cypermethrin exerted the most detrimental effect on oogenesis in *D. reticulatus* females. Under the effect of the 0.015625% solution, only 33.35% of females were able to lay eggs. Deltamethrin and cypermethrin disturbed egg maturation at higher concentrations. After application of pyrethroids, the oviposition period was prolonged 2-fold or 2.5-fold in the *D. reticulatus* females. By their effect on the nervous system, pyrethroids can regulate the release of hormones that play an important role in the tick oogenesis process [9]. Cypermethrin was shown to stimulate synthesis of vitellogenin in unengorged *Ornithodoros parkeri* (Cooley) by stimulation of release of the yolk production factor [10] and release of the vitellogenesis hormone [11]. Cypermethrin inhibits development of ovaries and decreases haemolymph concentrations of 20-hydroxyecdysone (20E, vitellogenesis hormone) and vitellogenin in engorged specimens, and causes degeneration of salivary glands inhibited by 20E in *O. parkeri* [12].

In turn, in *Amblyomma hebreum* Koch, cypermethrin inhibited egg development, in part probably due to inhibition of 20E release [10]. The pyrethroid tested in the presented study decreased the number and weight of *D. reticulatus* eggs and the egg conversion factor characterising the effectiveness of egg production. Egg batches treated with alpha-cypermethrin exhibited the lowest weight. Production of a larger number of eggs by the females and, hence, greater egg weight was associated with greater female weight loss.

Heavy metals [13] and phosphoorganic acaricides [14, 15, 16, 17] inhibited the number oogenesis in ticks, decreased the number of eggs laid by females, and decreased egg and larval survival. Inhibition of egg hatching was reported from *Rhipicephalus sanguineus*, which reached the levels of 72.1 and 42.0% when 0.01% cis-cypermethrin and 0.0025% deltamethrin were applied, respectively [18].

The biological effects of pyrethroids depended on the concentration of the tested substance. The increasing concentrations of all the tested pyrethroids raised the level of disturbances in the embryonic development which led to embryo death, usually before organogenesis. Deltamethrin application resulted in morphological anomalies in *D. reticulatus* larvae. As indicated by the investigations, deltamethrin had the most detrimental effect on embryos during the formation of blastoderm and embryonic streak, streak metamerisation and organogenesis (stage I and II), which resulted in the development of monstrosities in the larvae. Anomalies were observed in the walking legs, whose buds are formed in the earliest phase of embryogenesis, with the fourth pair degenerated at the end of the embryonic development, probably caused by apoptosis [19]. Similar anomalies in tick larvae were caused by chemical [20, 21, 22] and physical [23] agents.

The study shows that exposure of engorged ticks to pyrethroids efficiently reduces tick offspring abundance through their detrimental effect on maturation and development of eggs and the course of embryogenesis.

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