The effect of sublethal concentrations of deltamethrin and alphacypermethrin on the fecundity and development of *Ixodes ricinus* (Acari: Ixodidae) eggs and larvae

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Abstract

Two pyrethroids, deltamethrin (D) and alphacypermethrin (AC), have been used as commercial products for tick control worldwide. However, the effects of sublethal doses of these compounds on various tick species and their developmental stages have not been fully explored yet, although such knowledge could contribute to a more effective application of both pyrethroids and simultaneous reduction of their costs and undesirable side effects, including the harmful impact on other organisms and environmental contamination. This study investigates the effect of sublethal concentrations of D and AC applied to engorged females on the fecundity, development of eggs and larvae, and the number of offspring in the *Ixodes ricinus* (L.) tick, which is the most important vector of tick-borne diseases of humans in Europe. After detachment from rabbit's skin, fully engorged *I. ricinus* females were treated with 20 µl of pyrethroid solutions at five concentrations from 0.01562 to 0.25% and kept at 28 °C and 75% relative humidity. The impact of the pyrethroids on maturation and development of eggs as well as development of larvae was assessed based on parameters of the preoviposition and oviposition periods and the course of embryogenesis and egg hatch. The investigations have shown that both tested acaricides reduce the reproductive rate in *I. ricinus* females by inhibition of egg development and disturb embryonic development and larval hatch. Females did not lay eggs at concentrations higher than 0.0625% AC and 0.125% D. The lower concentrations of both pyrethroids disturbed or inhibited the embryogenesis and egg hatch in *I. ricinus*.

Keywords Alphacypermethrin · Deltamethrin · Pyrethroids · Vector control · Tick control · Tick reproduction

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Introduction

Given the direct effects of their parasitism and ability to transmit numerous pathogens, ticks represent arthropods with the greatest medical and epidemiological importance. The economic losses related to the detrimental effect exerted by ticks on livestock are very high (Jongejan and Uilenberg 2004; Ghosh et al. 2006; Ghosh and Nagar 2014). For instance, the annual expenditure related to ticks and animal tick-borne diseases are estimated at US\$ 3.24 billion in Brazil (Grisi et al. 2014), US\$ 573.61 million in Mexico (Rodríguez-Vivas et al. 2017), and US\$ 498.7 million in India (Minjauw and McLeod 2003). The costs of diagnostics and treatment of human tick-borne diseases, the incidence of which has risen in the recent decade, are substantial as well (Zhang et al. 2006; Müller et al. 2012). Therefore, investigations of the use of agents reducing tick population numbers in nature as control methods have aroused considerable interest.

The most common method for tick control is the use of acaricides, primarily pyrethroids that have been widely applied in the recent years. However, uncontrolled application of acaricides, disregarding the sensitivity of various developmental stages in individual species and the most efficient concentrations of the substances, contributes to development of resistance, which has been detected in some tick species (Mekonnen et al. 2002; Vatsya and Yadav 2011; Fernández-Salas et al. 2012; Abbas et al. 2014; Lenka et al. 2016) and in different populations of the same species (Sharma et al. 2012; Shyma et al. 2012; Abbas et al. 2014). Identification of the mode of action of different concentrations of chemical compounds against various tick species and their developmental stages is important for determination of their most effective dose reducing tick abundance and ensuring the lowest level of environmental contamination. The use of an appropriate acaricide dose is particularly important for the control of sympatric tick species, e.g. *Ixodes ricinus* (L.) and *Dermacentor reticulatus* (Fabricius) (Hubálek et al. 2003; Széll et al. 2006; Buczek and Bartosik 2011; Švehlová et al. 2014; Hofmeester et al. 2016; Stańczak et al. 2016; Olivieri et al. 2017; Radzijejskaja et al. 2018) and for minimisation of their harmful effects on other organisms present in the same ecosystem (Soderlund et al. 2002; Bradberry et al. 2005; Anadón et al. 2009; Antwi and Reddy 2015).

The aim of the present study was to determine remote effects of application of various concentrations of two pyrethroids, i.e. deltamethrin and alphacypermethrin, on engorged *I. ricinus* females and to establish the most efficient concentrations of the acaricides that will result in reduction of the abundance of tick offspring. Identification of the mechanism of action of acaricides on *I. ricinus* ticks has practical importance, as this species is widely distributed in Europe across forest and recreational areas, where it infests a variety of animal species and humans. *I. ricinus* plays the most prominent role in the transmission of many pathogens, e.g. *Borrelia burgdorferi* sensu lato and tick-borne encephalitis virus (Estrada-Peña and Jongejan 1999; Jensen et al. 2017). It also causes skin lesions and systemic reactions in the host (Bartosik et al. 2011a; Wilhelmsson et al. 2013), and mammalian meat allergy (alpha-gal syndrome) (Commins et al. 2011; Nuñez et al. 2011; Fischer et al. 2014).

Materials and methods

Tick maintenance

Unfed adult stages of *I. ricinus* were collected from vegetation by flagging in eastern Poland (Lubycza Królewska, 23°31'E 50°20'N) during the spring activity peak of this

species (from April to May). Prior to the application of the acaricides, the ticks were kept in glass containers at approximately 90% humidity in the laboratory. Only intact ticks that were active over a few post-collection days were used in the experiments. Subsequently, 15 females and 5 males were placed on shaved skin of an albino New Zealand rabbit (*Oryctolagus cuniculus*) kept at room temperature of ca. 20 °C and ca. 50% humidity. The presence of the males guaranteed fertilization of each female, since mating in this species can take place both away from the host and on the host. The course of feeding was assessed daily at the same time. Immediately after detachment from rabbit skin, each engorged *I. ricinus* female was weighed using a digital analytical laboratory balance with an accuracy of 0.01 mg (RADWAG XA WPA 120/C/1) and transferred to a rearing chamber lined with Whatman filter paper discs. 134 females and 45 males were used in the examinations. Rabbits used in the experiment were provided with drinking water and commercial pellet food ad libitum.

Test procedure

Just after detachment engorged *I. ricinus* females placed in separate rearing chambers were treated with 20 µl of the pyrethroid solutions using a 0.2–50 µl micropipette with an accuracy of 0.5–2%. The rearing chambers with the females were placed in the dark at a temperature of 28 °C and 75% RH before and during oviposition. As shown in our investigations, maintenance of a temperature of 28 °C during rearing of *I. ricinus* ticks, which prefer high humidity, reduces the risk of growth of mould fungi, which inhibit embryonic development of eggs and can lead to their death or the death of females before the end of oviposition (unpubl. data). After completion of oviposition, the *I. ricinus* females and eggs laid were weighed.

The course of embryonic development was assessed on the basis of the number of dead eggs, the number of dead embryos, and the number of larvae with developmental anomalies, larvae with hatching disturbances, and normal larvae, in accordance with criteria adopted in similar investigations of *D. reticulatus* (Buczek et al. 2013, 2014a). The same procedures as those employed in the acaricide treatment experiments were simultaneously applied in a control tick group, in which the females were treated with 20 µl of water instead of the tested substances.

In each experimental group receiving the different acaricide doses and in the control group, selected characteristic features of the non-parasitic stage of engorged females, including the preoviposition and oviposition periods and the course of embryonic development and *I. ricinus* egg hatch, were assessed. On the basis of the results obtained, such parameters as preoviposition period (PP), egg laying frequency (ELF), female postoviposition weight (FPW), female oviposition weight loss (FOWL), egg mass weight (EMW), egg conversion factor (ECF), hatching frequency (HF), embryogenesis period (EP), and hatching success (HS) were determined as in our previous studies on *D. reticulatus* (Buczek et al. 2013, 2014a).

Additionally, in each egg batch, the percentage of dead eggs, eggs with developmental disturbances at various embryogenesis stages, as well as the number of larvae with morphological anomalies, larvae with abnormal hatching, and normal larvae were determined (Buczek et al. 2013).

Tested acaricides

The activity of two synthetic pyrethroids, i.e. deltamethrin (D) (commercial name: K-Othrine 2.5 flow produced by Roussel Uclaf, France) and alphacypermethrin (AC) (commercial name: Alfasect 5SC, produced by ASPRAN s.c. Jaworzno, Poland), at the concentrations of 0.01562, 0.03125, 0.0625, 0.125, and 0.25% was tested (Table 1). The use of the same concentrations of the compounds as in our earlier studies on *D. reticulatus* facilitates comparison of the sensitivity of both tick species to these substances. The dose applied for one specimen contained in 20 µl of the solution was calculated based on the concentrations of the pyrethroid solutions tested (Table 1).

Statistical analysis

STATISTICA 5 and Microsoft Excel XP were used to analyse the results. 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.

Results

Eggs were laid by *I. ricinus* females treated with 0.01562–0.125% deltamethrin (doses 0.07812–0.625 µg/specimen) and 0.01562–0.0625% alphacypermethrin solutions (doses 0.15625–0.625 µg/specimen) (Figs. 1, 2). However, at the highest concentration of the AC, the eggs were deformed; probably due to the abnormal development of egg casings, which disintegrated and released egg content, the determination of the number of eggs in the batch was impossible. The proportion of females capable of egg production decreased with the increasing concentrations of the tested substances (Figs. 3, 4). At the highest concentrations of deltamethrin and alphacypermethrin that did not deprive the females of the oviposition ability, only 42.9 and 14.3% of females, respectively, laid eggs (100% in the control group).

The preoviposition period in *I. ricinus* was prolonged upon application of both pyrethroids. At the 0.01562% deltamethrin concentration (0.07812 µg/specimen), the eggs developed for 15.00 ± 7.823 days and at 0.125% as long as 28.333 ± 8.963 days (control 8.467 ± 1.196 days). The differences were statistically significant (Table 2). The H test confirmed the statistically significant differences in the length of the preoviposition period in the groups of females treated with the various concentrations of the active agent. Similar

Table 1 Quantity of active substance in 20 µl of a deltamethrin and alphacypermethrin solutions applied as a single dose (in µg)

Concentration of the solution (%)	Content of active substance in 20 µl of solution (in µg)	
	Deltamethrin	Alphacypermethrin
0.01562	0.07812	0.1562
0.03125	0.1562	0.3125
0.0625	0.3125	0.625
0.125	0.625	1.25
0.250	1.25	2.50

Fig. 1 Egg maturation and embryonic development in *Ixodes ricinus* under the influence of different concentrations of deltamethrin at 28 °C and 75% RH; *ELF* egg laying frequency, *HF* hatching frequency, *HS* hatching success

Fig. 2 Egg maturation and embryonic development in *Ixodes ricinus* under the influence of different concentrations of alphacypermethrin at 28 °C and 75% RH; *ELF* egg laying frequency, *HF* hatching frequency, *HS* hatching success

Fig. 3 Female and the egg batch of *Ixodes ricinus* after application of 0.01562% deltamethrin

Fig. 4 Female and the egg batch of *Ixodes ricinus* after application of 0.0625% deltamethrin

trends were found in the alphacypermethrin treatment. The differences in the length of the preoviposition period induced by the action of the 0.01562% and 0.03125% AC solution (0.1562 and 0.3125 $\mu\text{g}/\text{specimen}$, respectively) and that in the control group as well as the differences between the experimental groups treated with the different concentrations of AC were statistically significant (Table 2).

The mean number of eggs laid by the *I. ricinus* females decreased to 1664.5 and 1303.0 upon application of the lowest deltamethrin and alphacypermethrin concentrations, respectively (2615 eggs in the control group). The increasing concentrations of the substance reduced the number of eggs in the batch (Figs. 4, 5).

Deltamethrin concentrations higher than 0.125% (0.625 $\mu\text{g}/\text{specimen}$) and alphacypermethrin concentrations exceeding 0.03125% (0.3125 $\mu\text{g}/\text{specimen}$) inhibited the development of normal eggs. Both the egg mass weight and the egg conversion factor decreased

Table 2 Parameters of the eggs maturation and oviposition course in *Ixodes ricinus* females under the influence of different concentration of deltamethrin and alphacypermethrin at 28 °C and 75% RH

Chemical	Concentration (%)	PP (days) ^a			FPW (g) ^b			FOWL (%) ^c		
		M	SD	p	M	SD	p	M	SD	p
Deltamethrin N=45	0.01562	15	7.823	0.029	0.103	0.015	0.16	62.817	3.134	0.066
	0.03125	16.4	2.608	0.00041	0.155	0.033	0.0095	53.113	13.836	0.0054
	0.0625	17	1.000	0.0048	0.167	0.064	0.060	52.745	22.331	0.17
	0.125	28.3	8.963	0.0048	0.132	0.014	0.19	64.981	2.163	0.31
Alpha-cypermethrin N=39	0.01562	14.8	3.962	0.0017	0.133	0.023	0.12	45.988	16.637	0.0046
	0.03125	16.2	6.850	0.0018	0.167	0.067	0.12	45.818	20.560	0.087
	0.0625	25	0.000	1.0	0.184	0.000	1.0	31.860	0.000	1.0
	0.125	–	–	–	–	–	–	–	–	–
Control N=50		8.4	1.196	x	0.115	0.020	x	67.110	6.361	x

^aPreoviposition period^bFemale postoviposition weight (weight of female body after oviposition)^cFemale 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) *N* number of ticks used in the experiment, *M* mean, *SD* standard deviation

Fig. 5 Egg amount in *Ixodes ricinus* under the influence of different concentrations of deltamethrin and alphacypermethrin at 28 °C and 75% RH

Table 3 Parameters of the eggs maturation and oviposition course in *Ixodes ricinus* females under the influence of different concentration of deltamethrin and alphacypermethrin at 28 °C and 75% RH

Chemical	Concentration (%)	EMW (mg) ^a			ECF ^b		
		M	SD	p	M	SD	p
Deltamethrin N=45	0.01562	0.114	0.021	0.00082	0.415	0.093	0.030
	0.03125	0.108	0.027	0.00097	0.322	0.078	0.0011
	0.0625	0.087	0.049	0.0085	0.245	0.157	0.010
	0.125	0.081	0.042	0.020	0.219	0.121	0.024
	0.25	—	—	—	—	—	—
Alpha-cypermethrin N=39	0.01562	0.057	0.025	0.0	0.238	0.129	0.0028
	0.03125	0.047	0.035	0.0	0.145	0.099	0.0016
	0.0625	0.034	0.000	0.0	0.127	0.00	0.0
	0.125	—	—	—	—	—	—
	0.25	—	—	—	—	—	—
Control N=50		0.187	0.031	x	0.537	0.109	x

^aEgg mass weight—the total weight of eggs laid by a female

^bEgg conversion factor—engorged female weight used for egg production, *N* number of ticks used in the experiment, *M* mean, *SD* standard deviation

statistically significantly compared with the control group and decreased with the increasing concentration of the two tested substances (Table 3). The different concentrations of deltamethrin and alphacypermethrin caused statistically significant changes in the female postoviposition weight and in the indicator of female oviposition weight loss (Table 2).

The pyrethroids tested disturbed the course of the embryonic development of *I. ricinus* eggs (Table 4). Statistically significant differences in the length of the embryonic development period were found upon application of 0.03125% deltamethrin as well as 0.01562 and 0.031255% alphacypermethrin, compared with the control (Table 4). The H test revealed a statistically significant difference in the length of embryogenesis between the experimental groups treated with the different alphacypermethrin concentrations.

Normal larvae only hatched in experiments in which *I. ricinus* females were treated with the 0.01562 and 0.03125% deltamethrin solutions (67.07 and 41.14% of larvae,

respectively; control 89.64%). In these experimental conditions, the embryonic development of a substantial proportion of eggs was impaired at various stages, and egg death in embryogenesis stage I, II, and III, larvae trapped in the egg casing (Fig. 6), and larvae with morphological anomalies were noted (Table 5). Even more serious disturbance in the course of the initial stage of embryonic development was observed when the higher deltamethrin concentrations were applied. No normal larvae hatched and all eggs died soon after oviposition as a result of application of each alphacypermethrin concentration.

Discussion

Ixodes ricinus is a three-host species with a 2.5–3-year-long developmental cycle in the temperate climate zone (Chmela 1969). The biological features of *I. ricinus*, i.e. high fertility (Bartosik and Buczek 2012), an ability to adapt to various types of habitats and abiotic conditions (Bartosik et al. 2011b; Geller et al. 2013; Pangráčová et al. 2013; Buczek et al. 2014b), and a wide host range including numerous small and big mammals and birds (Gern 2008), contribute to changes in their distribution range and increased abundance of this tick species (Jaenson et al. 2012; Medlock et al. 2013). The expansion of the *I. ricinus* occurrence range was facilitated by climatic and weather changes as well as human activities influencing groups of plants and animals that are potential hosts of various developmental stages of this tick species (Estrada-Peña et al. 2006; Gray 2008; Schwarz et al. 2009; Buczek et al. 2014b).

Application of effective acaricides at time intervals controlled by environmental monitoring over a given area may substantially reduce the abundance of the tick species (Benelli et al. 2017), thereby mitigating the risk of tick-borne diseases (Pegram and Eddy 2003; Otranto et al. 2010). However, application of acaricides may lead to development of tick cross-resistance to chemical compounds with a similar mode of action or even multiple resistance to several chemical compounds with different mechanisms of activity (Sutherst and Comins 1979; George et al. 2004; Abbas et al. 2014). The development of acaricide

Table 4 Embryogenesis period in *Ixodes ricinus* under the influence of different concentration of deltamethrin and alphacypermethrin at 28 °C and 75% RH

Chemical	Concentration (%)	EP (days) ^a		
		M	SD	p
Deltamethrin N = 45	0.01562	33.2	7.259	0.14
	0.03125	32.6	1.949	0.038
	0.0625	31	1.000	0.38
	0.125	30.5	3.536	0.82
	0.25	–	–	–
Alphacypermethrin N = 39	0.01562	35.7	5.909	0.022
	0.03125	37.5	6.028	0.013
	0.0625	36	0.00	1.0
	0.125	–	–	–
	0.25	–	–	–
Control N = 50		29.5	3.361	–

^aEmbryogenesis period: period between the beginning of oviposition to hatching of the first larva, *N* number of ticks used in the experiment, *M* mean, *SD* standard deviation

Fig. 6 *Ixodes ricinus* larva trapped in the egg casing

resistance in ticks is influenced by many factors, e.g. the structure and dose of chemicals, mode of application of the formulations, frequent use of the same tick control product for a long time, and use of the chemical substances for other purposes, e.g. for pest control. Given the possibility of resistance in ticks, the use of sublethal acaricide doses, similar to those tested in this study, should be monitored and resistance management practices with integrated actions in tick control should be applied in the area of where the chemicals are used (Kunz and Kemp 1994; Ghosh et al. 2006; Stafford et al. 2017). In turn, reduced concentrations of acaricides introduced to tick habitats and/or applied to tick hosts increase the degree of environmental contamination and the negative effect on other organisms (Kunz and Kemp 1994; Soderlund et al. 2002; Das and Mukherjee 2003; Antwi and Reddy 2015; Glorennec et al. 2017). The use of a reduced amount of active substances in formulations has economic importance as well, as it lowers the high costs of the control of ticks and tick-borne diseases (Ostfeld et al. 2006; Stafford et al. 2017). Acaricides with various chemical structures and different toxicities have been used for *I. ricinus* control on the host (e.g. Henderson and Stevens 1987; Taylor and Elliott 1987; Mehlhorn et al. 2011; Wengenmayer et al. 2014) and away from the host (e.g. Rupeš et al. 1972; Bogachkina et al. 2011). However, only few investigations were focused on assessment of the effects of application of sublethal pyrethroid doses on female reproductive performance and embryonic development in this species (Buczek et al. 2014c). Such investigations are particularly advisable since, due to the slow rate of development of tick poisoning, unfed ticks are able to infest a host and ingest its blood, engorged females are capable of oviposition, and engorged young stages are able to moult into successive developmental stages. The phenomenon of “overcoming the poisoning” in ticks may contribute to sustenance of interspecific and intraspecific pathogen transmission (Uspensky and Ioffe-Uspensky 2006).

Our investigations indicate that the detrimental effect of the tested deltamethrin and alphacypermethrin pyrethroids applied during the preoviposition period can markedly reduce the abundance of *I. ricinus* offspring. The treatment of engorged females even with the lowest 0.01562% solutions of deltamethrin (0.07812 µg/specimen) and alphacypermethrin (0.1562 µg/specimen) extended the preoviposition period 1.78- and 1.76-fold, respectively, compared with the control. The highest concentrations of the tested substances, i.e.

Table 5 Course of embryonic development in *Ixodes ricinus* under the influence of deltamethrin and alphacypermethrin at 28 °C and 75% RH

Parameter	Deltamethrin concentration (%)		Alphacypermethrin concentration (%)		Control N = 15,690		
	0.01562 N = 9987	0.03125 N = 10,863	0.0625 N = 4890	0.125 N = 3372		0.01562 N = 7018	0.03125 N = 2598
Normal larvae (%)	67.07	41.14	0.00	0.00	0.00	0.00	89.64
Larvae with developmental anomalies (%)	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Larvae with hatching disturbances (%)	2.97	0.97	1.72	0.00	0.00	0.00	0.12
Dead eggs (%)	1.08	0.33	1.10	3.02	0.23	0.00	0.35
Inhibition in embryogenesis (%)	28.83	57.57	97.18	96.98	99.77	100	9.88
In the I stage (%)	60.35	92.17	97.22	100.00	100	100	54.13
In the II stage (%)	35.60	6.39	2.78	0.00	0.00	0.00	35.67
In the III stage (%)	4.05	1.43	0.00	0.00	0.00	0.00	10.20

N—number of *Ixodes ricinus* eggs included in six egg batches analyzed to determine the course of embryonic development and larval hatch under the influence of different permethrin concentrations

0.125% deltamethrin (0.625 µg/specimen) and 0.0625% alphacypermethrin (0.625 µg/specimen), lead to even more pronounced extension, 3.3- and 2.9-fold respectively, of the pre-oviposition period. Due to the disturbed course of maturation and egg development, a lower number of females were capable of oviposition, and the number of eggs in batches declined considerably. In this stage of the *I. ricinus* life cycle, alphacypermethrin proved to be a potent agent, whose lowest concentrations of 0.01562% (0.1562 µg/specimen) decreased the number of eggs in batches by half, whereas the concentration of 0.03125% (0.3125 µg/specimen) caused a six-fold reduction compared with the control. On the average, the egg mass weight decreased over three times and the egg conversion factor declined over two times when the females were treated with alphacypermethrin. Davey et al. (1998) reported that another pyrethroid fipronil reduced the weight of feeding females and their eggs in the one-host tick *Rhipicephalus (Boophilus) microplus* (Canestrini).

Our results correspond with the investigations carried out by other authors (Friesen and Kaufman 2003; Oliveira et al. 2008; Roma et al. 2010; Camargo-Mathias et al. 2017), who demonstrated cytotoxic effects of pyrethroids on the tick reproductive system. Cypermethrin inhibited oocyte development in *Amblyomma hebraeum* Koch (Friesen and Kaufman 2003). In semi-engorged *Rhipicephalus sanguineus* (Latreille), fipronil induced changes in the number and size of oocytes, as well as changes in their structure (cytoplasm vacuolisation) (Oliveira et al. 2008). Similarly, in this species, permethrin induced a decrease in the oocyte size, appearance of large vacuoles in their cytoplasm, and a decline in the number of yolk granules (Roma et al. 2010).

As suggested by Balashov (1983) in a study of *Hyalomma asiaticum* Schulze et Schlottke, there are two (endogenous and exogenous) sources of yolk production in ticks. The ultrastructural analysis of *R. sanguineus* ovary shows that endogenous yolk synthesis begins in oocytes II, and the number of yolk granules increases in the successive stages of oocyte development (III–IV) (Oliveira et al. 2005). In turn, the exogenous sources of yolk probably include both the fat body (in whose cells vitellogenic proteins are secreted into the hemolymph) (Sonenshine 1991) and the pedicel cells (Oliveira et al. 2005).

Through their neurotoxic effect on ticks (Roma et al. 2010, 2013), pyrethroids impair synthesis and/or secretion of hormones regulating vitellogenin synthesis in argasid (Chinzei et al. 1989; Taylor et al. 1991) and ixodid ticks (Friesen and Kaufman 2002, 2003; Roma et al. 2012).

The substances disturbed the embryonic development in *I. ricinus* more severely, resulting in prolongation of this period and inhibition of the first stage of embryogenesis, as well as inhibited larval hatch and development of abnormal larvae.

The morphological anomalies in larval legs of *I. ricinus* such as oligomely (lack of appendages) or fusion of appendages on the same side of the idiosoma and the presence of larvae with hatch disturbances reported in the present study are similar to those found in ticks collected in Upper Silesia, a region highly contaminated with industrial waste (unpublished data), and those reared in laboratory conditions at various temperature ranges (Buczek 1992) and humidity conditions (Buczek 2000). In *D. reticulatus* tick larvae, morphological changes affecting walking legs, e.g. oligomely, leg fusion, or branching legs, were also induced by deltamethrin (Buczek et al. 2013) and permethrin (Buczek et al. 2014a). An interesting phenomenon of formation of an additional (fourth) pair of legs in *D. silvarum* Olenov hatched from eggs laid by females treated with DDT was reported by Ioffe (1984).

The results of these studies imply a role of the nitrile group (enhancing the amphiphilic nature of linkages) and halogen in the vinyl substituent in the pronounced teratologic effect of the pyrethroid on *I. ricinus*. It seems possible that, irrespective of the difference in the

internal energy of the stereoisomeric forms, the type of the halogen determines the efficiency of the cellular of N-alkylation processes. The alphacypermethrin molecule contains chlorine, whereas the deltamethrin molecule—bromine.

As in the case of *I. ricinus*, alphacypermethrin exerted a more potent toxic effect than deltamethrin on the development of eggs and larvae in *D. reticulatus*, another Palearctic tick species. However, a comparison of the current study and previous results obtained simultaneously (Buczek et al. 2013, 2014c) shows that the activity of the tested agents differs in both tick species. Treated with the same deltamethrin doses at all the concentrations ranging from 0.01562 to 0.125%, fewer *I. ricinus* females laid eggs, compared with *D. reticulatus* females. In turn, the lowest doses of alphacypermethrin reduced the reproductive performance more efficiently in *D. reticulatus* than in *I. ricinus* females; however, it completely inhibited maturation and egg development only at the concentration of 0.25% (Buczek et al. 2014c).

Likewise in *I. ricinus*, alphacypermethrin inhibited egg embryonic development and larval hatch in *D. reticulatus* at the lowest 0.01562% concentrations (Buczek et al. 2013). The course of embryonic development upon application of deltamethrin differed in these species, hence the different proportions of dead eggs, abnormally hatched larvae, and normal larvae. In *I. ricinus*, 41.14% of normal larvae hatched at the 0.03125% concentration of deltamethrin and complete inhibition of larval hatch was noted at the concentration of 0.0625% of this substance. 0.01% cis-cypermethrin, 0.015% cypermethrin, and 0.0025% deltamethrin inhibited larval hatch in *R. sanguineus* in 72.1, 67.3, and 42.0%, respectively (Bicalho et al. 2001).

The different effects of deltamethrin and alphacypermethrin on *I. ricinus* and *D. reticulatus* corroborate the need for assessment of the acaricidal effects of chemical compounds in various tick species. Uspensky and Ioffe-Uspensky (2006) found that the duration of poisoning development in adult tick females from several genera of ticks after acaricide application correlated with the degree of species-specific refractoriness. Tick age also exerts an effect on their sensitivity to chemical substances (Rupeš et al. 1972; Mount 1984; Uspensky and Ioffe-Uspensky 2006).

Conclusion

The investigations indicate that application of the tested compounds in appropriate sublethal doses can reduce the abundance of successive generations of *I. ricinus*, which may contribute to reduction of pathogen transmission in the population of this tick species. Given the necessity of minimisation of the toxic effects of chemical substances on the environment and reduction of the high costs of tick control, lower doses of alphacypermethrin and deltamethrin decreasing the abundance of tick offspring can be applied in *I. ricinus* control. Yet, this practice offers a possibility of development of tick resistance to these synthetic pyrethroids. Hence, in areas where low doses of synthetic pyrethroids are applied in tick control, it is particularly essential to monitor the efficacy of the chemicals and rigorous resistance management. It is also indispensable to develop a strategy for control of ticks and tick-borne diseases.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (the study was approved from the Local Commission for Animal Experiments, Lublin, Poland).

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