Short Communication

Circadian Changes in Susceptibility of Various Species of *Gryllidae* to Insecticides, Depending on Time of Intoxication and Size of Tested Group

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Abstract

Circadian changes in the susceptibility of adult individuals of three cricket species: field cricket (*Gryllus campestris*), house cricket (*Acheta domesticus*), and tropical house cricket (*Gryllodes sigillatus*) to insecticides belonging to the group of synthetic pyrethroids (Bulldock 025 EC) and oxadiazine (Steward 30 WG) were tested in 2007 and 2008. The assessment included the intoxication of subsequent groups of animals with insecticides at four crucial times of the diurnal cycle – at sunrise, at noon, at sunset, and at midnight. Due to the strongly developed territorial behaviour of *Gryllidae*, which may strongly affect the results of the analysis, tests were performed both on animals kept separately and on groups of 10 individuals. The application of Steward 30 WG and Bulldock 025 EC caused a significant decrease in the animals' survival rate to the level of 41% (intoxication at midnight) and 38% (intoxication at sunrise) in *G. campestris*; 50% (intoxication at sunset) and 61% (intoxication at midnight) in *A. domesticus*; and 45% (intoxication at midnight) and 50% (intoxication at sunrise) in *G. sigillatus*, respectively. In the case of *G. campestris* (the control group) and *G. sigillatus* (group treated with Bulldock 025 EC) the size of tested groups had a significant influence on their survival rate.

Keywords: *Gryllidae*, cricket, circadian rhythm, insecticide

Introduction

Crickets (*Gryllidae*) are often considered to be synanthropic species [1, 2]. Due to a strongly developed territorial behaviour [3-6] which prevents an overexpansion of their population they produce loud sounds (reaching 100 dB) which may cause a particular kind of discomfort in humans [7, 8]. In spite of this, they are not regarded as pests [7]. However, the disadvantages mentioned above may cause more serious problems, especially in the case of the tropical species of crickets, including the house cricket (*Acheta domesticus*), commonly found in Poland, or the more and more common tropical house cricket (*Gryllodes sigillatus*) [9], since under favorable conditions of human housing they are able to perform mating activity throughout the whole year (personal observations). To make matters worse, these cricket species are omnivorous. They feed not only on food waste produced by humans, but also clothing made up of natural fabrics. Moreover, they eat skin and fur, and contaminate the habitat by their droppings and moults.

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The application of plant protection agents in the places of continuous human habitat is always a hazardous venture as these preparations are not neutral to mammals [10-15]. Testing the effect of the time of intoxication on the susceptibility to insecticides may help to establish the proper time for chemical substances application, so that the insecticidal action is most effective. The aim of the following study was to investigate to what extent the time of day, when the contact of toxic chemical with tested cricket occurred, could modify the effect of the xenobiotic on the insect's body, and whether the size of a tested group could be significant.

Material and Methods

Animals

2880 imago forms of both sexes (1:1) of house cricket (*A. domesticus*), field cricket (*G. campestris*), and tropical house cricket (*G. sigillatus*) originating from a breeding colony of the Department of Ecotoxicology UR were used in the research. While in breeding colony, animals were subjected to fixed thermal conditions 27.0±1.0ºC and natural light regime.

Chemicals

The aqueous solutions of the following insecticides were used:

Steward 30 WG. Producer: Du Pont de Nemours. Active ingredients: indoxacarb – 300 g in 1 kg of the agent. The concentration of the usable liquid 2 g/L (*G. campestris*, *A. domesticus*, and *G. sigillatus*),

Bulldock 25 EC. Producer: Irvita Plant Protection N.V. Active ingredients: beta-cyfluthrin – 25 g in 1 liter of the agent. The concentration of the usable liquid 0.2 g/L (*G. campestris* and *A. domesticus*) and 0.18 g/L (*G. sigillatus*).

Experimental Procedure

Laboratory tests checking the influence of insecticides on the survival rate of crickets were carried out in April (*G. campestris*) and October (*A. domesticus* and *G. sigillatus*) 2007 and 2008 (six repetitions each time). Experiments were conducted in fixed temperature of 23ºC using the natural diurnal rhythm of light and darkness. Animals had unlimited access to food and water. Two types of tests were performed. In the first one, the animals were kept in isolation from other individuals of the same species during the whole test (the single test), in the second type of tests they were collected in groups of 10 animals (the group test). In both tests the number of individuals or groups used in one repetition was 10.

Chronotoxicological experiments commenced at four crucial times of the day: at sunrise, at noon, at sunset, and at midnight. The established duration of each experimental cycle was three days, starting from the first contact of an animal with a preparation.

Table 1. List of statistically significant differences in survival rate of three species of cricket depending upon the size of the group.

Species	Compound	Time	Level of significance
G. campestris	control	sunrise	p<0.001
G. sigillatus	Bulldock 025 EC	sunrise	p<0.05

A 4 µl drop of preparation (water – in the control group) was applied on the ventral part of the insect's thorax, near the suboesophageal ring, with the use of an automatic pipette. After 20 seconds, unabsorbed volume of the substance was removed with a blotting paper.

The statistical analysis was carried out using Statistica software, version 10.0. The survivability of insects was determined using one-way ANOVA with the time of the day or the size of the group as main factors. Statistical significance of individual differences was determined using Tukey's post-hoc test.

Results

Figs. 1-3 present the effects of the time of day when the intoxication occurred (sunrise, midday, sunset, midnight) and the number of animals in tested groups on the survival rate, within 72 hours from the contact of animals with tested insecticides, in individuals belonging to three different species of *Gryllidae*.

In general, pesticides used in the study caused a reduction in cricket survival rates, both in the single tests and in the group tests (Figs. 1, 2, 3, Table 2). The only exception was *G. campestris*. During the single tests after the intoxication with insecticides its survival rate was similar to the one obtained in other studied cricket species (Figs. 1b, 1c). However, in this case in the control group the survival rate in the group test was much lower than that in the single test (Fig. 1a, Table 1). Moreover, only in the case of this species, a significant influence of the time of the day when the intoxication occurred on the survival rate of control animals was observed.

In animals treated with Steward 30 WG and Bulldock 025 EC (Figs. 1b, 1c, 2b, 2c, 3b, 3c) the susceptibility to insecticides was variable, depending on the phase of circadian rhythm, when these substances were applied. Therefore, in all the cases, animals intoxicated during the day (especially at noon) showed a higher survival rate than those treated with insecticides at other times of the day. In crickets intoxicated with Steward 30 WG (Figs. 1b, 2b, 3b), in the group tests, the survival rate was slightly higher than in the single tests. Bulldock 025 EC (Figs. 1c, 2c, 3c) had an opposite effect – animals kept separately showed higher survival rates after contact with this substance, compared to the animals tested in groups.

Only in the case of *G. sigillatus* was the survival rate after intoxication with the pesticide dependent on the size of the group (Fig. 3 Table 1).

Discussion

Our experiments showed that Steward 30 WG and Bulldock 025 EC were least toxic during the lowest activity of insects, which confirms our previous research carried out on *Leptinotarsa decemlineata* [16] and on young [17] and old worker bees *Apis mellifera* [18]. It seems likely that in the case of all studied species, adjusting the time of insecticide application into the fluctuation of the animal's activity may have a beneficial effect on their selective elimination (reducing harm to other species). Conversely, particularly in the case of honeybees, this mode of action may contribute to their protection against poisoning.

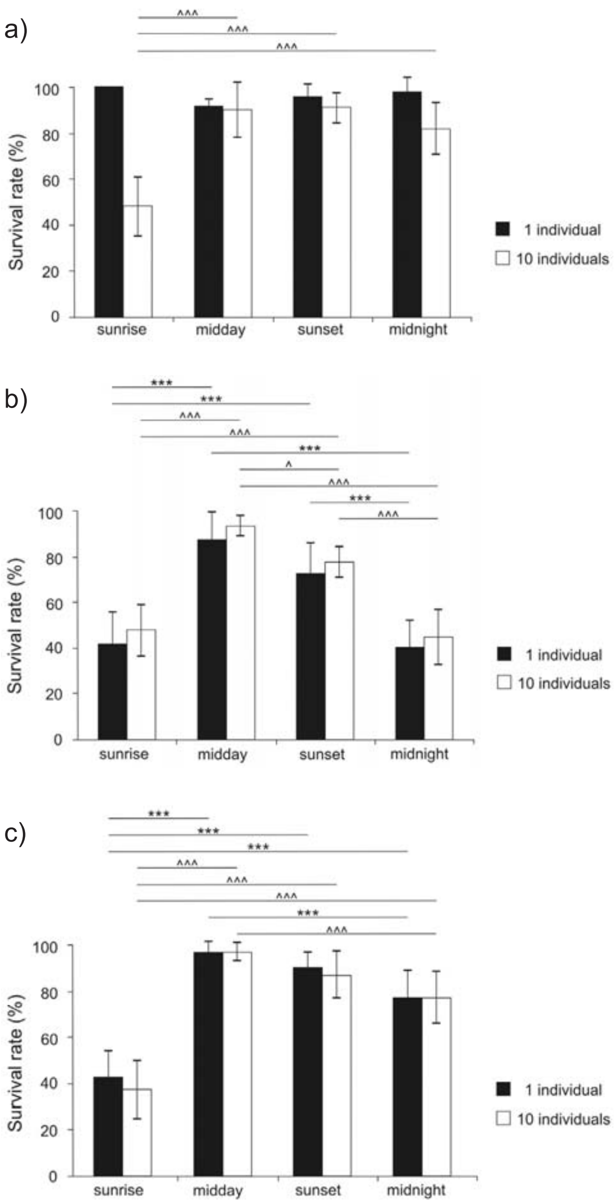

Fig. 1. Mean survival rate of adult individuals of field cricket (*G. campestris*) depending upon the time of intoxication and size of tested group. Panel a – control, Panel b – Steward 30 WG, Panel c – Bulldock 025 EC.
Statistically significant differences in the case of one individual were marked as: *p<0.05, **p<0.01, ***p<0.001;
Statistically significant differences in the case of the group of 10 individuals were marked as ^p<0.05, ^^p<0.01, ^^^p<0.001.

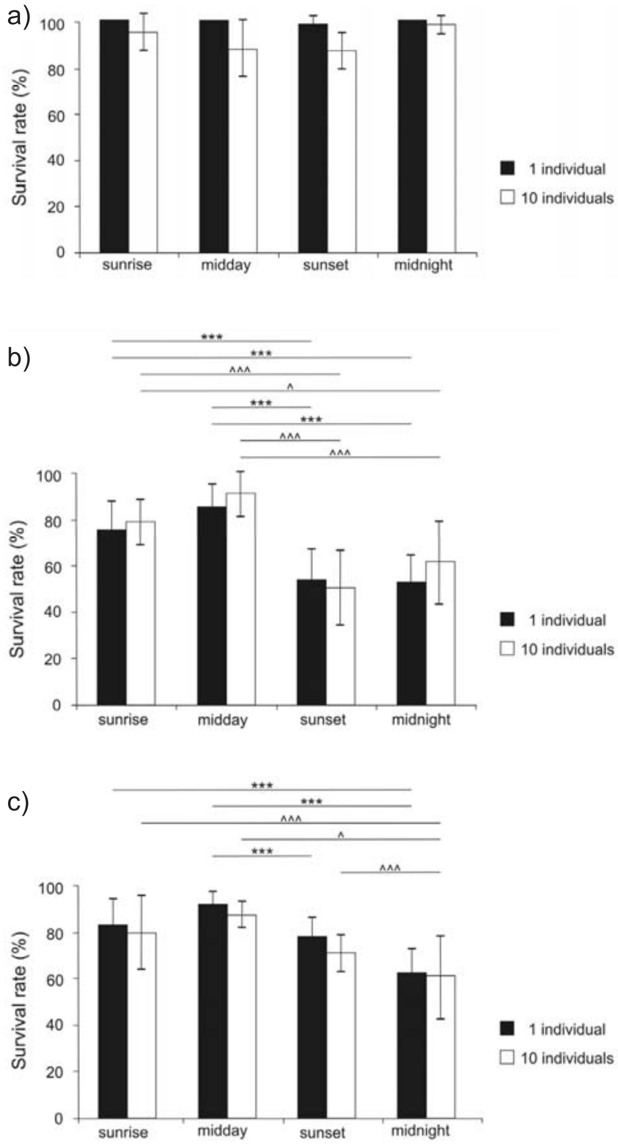

Fig. 2. Mean survival rate of adult individuals of house cricket (*A. domesticus*) depending upon the time of intoxication and size of tested group. Panel a – control, Panel b – Steward 30 WG, Panel c – Bulldock 025 EC.
Statistically significant differences in the case of one individual were marked as: *p<0.05, **p<0.01, ***p<0.001;
Statistically significant differences in the case of the group of 10 individuals were marked as ^p<0.05, ^^p<0.01, ^^^p<0.001.

Also, the size of the tested group may influence the survival rate of animals. This phenomenon is explicitly visible mainly in the case of *G. campestris* – the biggest and the most aggressive individuals of the same species among all the tested crickets. *Gryllidae* males are characterized by a significant territoriality. They are extremely aggressive against other individuals of its species, especially when thermal conditions are forcing a procreative behaviour, and also because of the presence of females [19]. That is why, in the control group consisting of 10 individuals, the most important factor determining survivability was inter-individual interaction. In such groups, only females survived until the end of the experiment because the males were fighting each other. Such a phenomenon was not observed

Table 2. List of statistically significant differences in survival rates between control and intoxicated representatives of three cricket species depending upon intoxication time.

	Size of group	Time	Level of significance		
			G. campestris	*A. domesticus*	*G. sigillatus*
Steward 30 WG	1	sunrise	p<0.001	p<0.001	p<0.001
Bulldock 025 EC	1	sunrise	p<0.001	p<0.05	p<0.001
Steward 30 WG	10	sunrise	-	p<0.05	p<0.001
Bulldock 025 EC	10	sunrise	-	-	p<0.001
Steward 30 WG	1	midday	-	p<0.05	p<0.001
Bulldock 025 EC	1	midday	-	-	p<0.01
Steward 30 WG	10	midday	-	-	p<0.001
Bulldock 025 EC	10	midday	-	-	p<0.01
Steward 30 WG	1	sunset	p<0.001	p<0.001	p<0.001
Bulldock 025 EC	1	sunset	-	p<0.001	p<0.001
Steward 30 WG	10	sunset	-	p<0.001	p<0.001
Bulldock 025 EC	10	sunset	-	p<0.05	p<0.001
Steward 30 WG	1	midnight	p<0.001	p<0.001	p<0.001
Bulldock 025 EC	1	midnight	p<0.01	p<0.001	p<0.001
Steward 30 WG	10	midnight	p<0.001	p<0.001	p<0.001
Bulldock 025 EC	10	midnight	-	p<0.001	p<0.001

to such an extent in intoxicated groups because animals were paralyzed to a greater or lesser degree by neurotoxins applied to them. This way, paradoxically, insects were protected against biting one individual by another because the competitors were paralyzed or the transmission of stimuli in their bodies was impaired [20]. *Gryllus campestris* are big crickets that are extremely aggressive toward individuals belonging to the same species [21]. Males, which are larger and have more symmetrical shapes, usually tend to achieve greater reproductive success [22, 23]. Such a phenomenon was not observed in *G. sigillatus*, where the main factor determining its attractiveness to females was the sound emitted, but not the male's size [21]. So the competitiveness in this species is linked to an aggression to a lesser extent, but to a greater extent to the stridulation. *A. domesticus* is also less aggressive toward individuals of the same species (personal observations). It is likely that the possibility of rapid energy loss by the cricket which is much smaller than in *G. campestris* is responsible for this situation [24]. In those crickets the victory in the fight against other individuals of the same species is due to determination in the fight rather than an individual's size [25].

We suppose that at least in the case of insects showing distinct aggression toward the representatives of its own species, the size of a tested group can significantly affect the final result of the experiment. As the aggression of *Gryllidae* males is changing in the presence of females [3, 5], experiments on separated animals could give different results than experiments on groups, depending on the size of the group and the sex distribution. However, this kind of experiment has not yet been conducted.

Steward 30 WG is the preparation whose total effect on survival rate is modified by the size of the tested group. This insecticide causes blocking the conduction of action potentials in excitable cell membranes by increasing the period of sodium channels' closed state [20, 14], thereby reducing survivability. On the other hand, in the group of a bigger size Steward 30 WG causes an increase in the survival rate compared to tests on animals kept separately.

Conversely, under the same conditions Bulldock 025 EC, which induces lowering of the threshold of the action potential generation [26, 13] causes a reduction in cricket survivability. Consequently, it may be assumed that when choosing agents to eliminate *Gryllidae*, the population density among potential environmental stressors should be taken into consideration.

Plant protection agents are harmful not only to organisms they are used against but also to humans. Recent decades have shown that pests, especially those belonging to the class of insects, very quickly become resistant to insecticides used against them. Hence searching for new preparations has been intensified and procedures of currently used biocides have been improved. The use of integrated plant protection schemes based on combining different methods of pest elimination is becoming more and more popular. Our previous studies [16-18] indicate that in the process of integrated plant protection the natural rhythm of both pests and beneficial insects sharing the same area

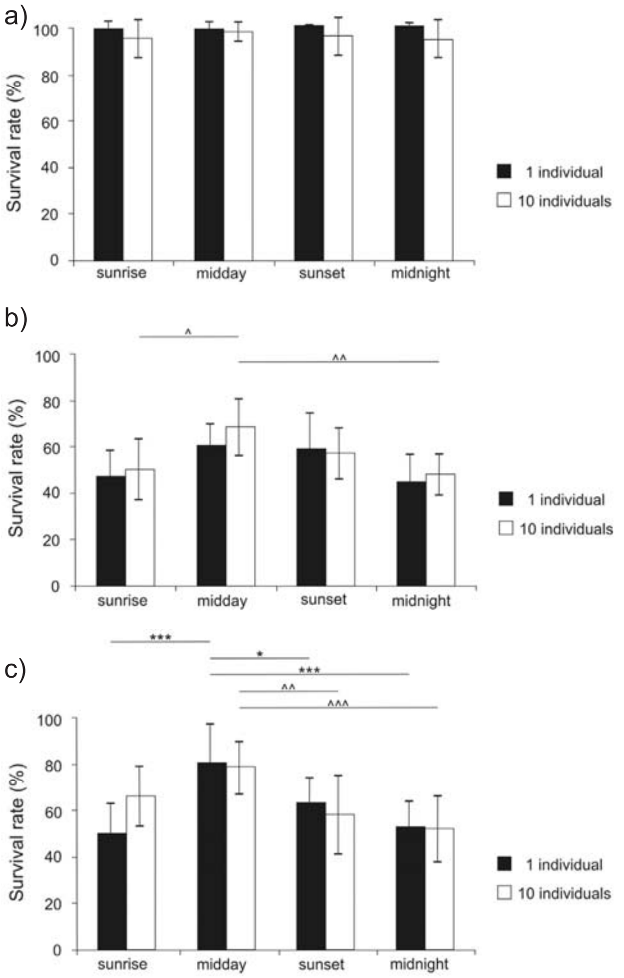

Fig. 3. Mean survival rate of adult individuals of tropical house cricket (*G. sigillatus*) depending upon the time of intoxication and size of tested group. Panel a – control; Panel b – Steward 30 WG; Panel c – Bulldock 025 EC.
Statistically significant differences in the case of one individual were marked as: *p<0.05, **p<0.01, ***p<0.001;
Statistically significant differences in the case of the group of 10 individuals were marked as ^p<0.05, ^^p<0.01, ^^^p<0.001.

should be considered. This would allow increasing the efficiency of pest elimination with the simultaneous limitation of the access to biocides of such organisms as *A. mellifera*, which should not be in contact with toxic substances.

Conclusion

1. In *Gryllidae* the susceptibility against insecticides varies in a 24 hour period.
2. Only in the case of *G. sigilatus* does the size of a tested group affect susceptibility to insecticides used in research.

References

1. BOGDANOVA E. Medically important synanthropic arthropods of Russia and neighboring countries. In: ROBINSON W. H., BAJOMI D. (Eds). Proceedings of the Sixth International Conference on Urban Pests. OOK-Press Kft: Hungary, pp. 355-360, **2008**.
2. WITTENBERG R., KENIS M., HÄNGGI A., GASSMANN A., WEBER E. Invasive alien species in Switzerland. An inventory of alien species and their threat to biodiversity and economy in Switzerland. FOEN Documentation: Bern, pp. 155, **2006**.
3. ADAMO S. A., HOY R. R. Agonistic behaviour in male and female field crickets *Gryllus bimaculatus*, and how behavioural context influences its expression. Anim. Behav. **49**, 1491, **1995**.
4. STANFORD F. Intra- and interspecific aggression in two species of field crickets: *Gryllus integer* and *G. alogus*. Aggressive Behav. **13**, (3), 149, **1987**.
5. WYNN H., VAHED K. Male *Gryllus bimaculatus* guard females to delay them from mating with rival males and to obtain repeated copulations. J. Insect Behav. **17**, (1), 53, **2004**.
6. ZUK M., SIMMONS L. W. Reproductive strategies of the crickets. (*Orthoptera*: *Gryllidae*). In: CHOE J. C., CRESPI B. J. (Eds.). The evolution of mating systems in insects and arachnids. Cambridge Univ. Press: Cambridge, pp. 89-109, **1997**.
7. POULET J. F. A. Corollary discharge inhibition and audition in the stridulating cricket. J. Comp. Physiol. A **191**, (11), 979, **2005**.
8. JONES M. D. R., DAMBACH M. Response to sound in crickets without tympanal organs (*Gryllus campestris* L.). J. Comp. Physiol. A **87**, 89, **1973**.
9. SCHMIDT G. R. New records of Grylloidea from Africa and the Indian subcontinent (Insecta: *Orthopteroidea*: *Ensifera*). J. Entomol. Res. Soc. **1**, (1), 39, **1988**.
10. EL-MORSI D. A., ABDEL RAHMAN R. H., ABOU ARAB A. A. K. Pesticides residues in Egyptian diabetic children: A preliminary study. J. Clinic. Toxicol. **2**, (6), 1000138, **2012**.
11. EL-HELALY M., ABDEL-ELAH K., HAUSSEIN A., SHALBY H. Paternal occupational exposures and the risk of congenital malformations – a case-control study. Int. J. Occup. Med. Environ. Health **24**, (2), 218, **2011**.
12. PIERIS-JOHN R. J., WICKREMASINGHE R. Impact of low-level exposure to organophosphates on human reproduction and survival. T. Roy. Soc. Trop. Med. H. **102**, (3), 239, **2008**.
13. SODERLUND D., CLARK J. M., SHEETS L. P., MULLIN L. S., PICCIRILLO V. J., SARGENT D., STEWENS J. T., WEINER M. L. Mechanism of pyrethroid neurotoxicity: implications for cumulative risk assessment. Toxicology **171**, 3, **2002**.
14. NARAHASHI T. Nerve membrane ion channels as the target site of insecticides. Mini Rev. Med. Chem. **2**, (4), 419, **2002**.
15. RECIO R., ROBBINS W. A., OCAMPO-GÓMEZ G. V., BORJA-ABURTO J., MORÁN-MARTINEZ J., FROINES J. R., GARCIA-HERNÁNDEZ R. M., CEBRIÁN M. E. Organophosphorous pesticide exposure increases the frequency of sperm sex null aneuploidy. Environ. Health Persp. **109**, (12), 1237, **2001**.
16. PIECHOWICZ B., STAWARCZYK K., STAWARCZYK M. Circadian changes of susceptibility to insecticides in colorado potato beetle (*Leptinotarsa decemlineata* Say) observed in laboratory and half-natural conditions. Zeszyty Naukowe Południowo-Wschodniego Oddziału Polskiego Towarzystwa Inżynierii Ekologicznej i Polskiego Towarzystwa Gleboznawczego w Rzeszowie **14**, 51, **2012** [In Polish].

17. PIECHOWICZ B., STAWARCZYK K., STAWARCZYK M. Circadian changes in susceptibility of young honeybee workers to intoxication by pyrethroid, carbamate, organophosphorus, benzoyl urea and pyridine derivative insecticides. J. Plant Prot. Res. **52**, (2), 286, **2012**.
18. PIECHOWICZ B., GRODZICKI P., STAWARCZYK M., STAWARCZYK K. Circadian and seasonal changes in the honeybee (*Apis mellifera*) workers susceptibility to diazinon, teflubenzuron, pirimicarb and indoxacarb. Pol. J. Environ. Stud. **22**, (5), 1457, **2013**.
19. KRAVITZ A. E., HUBERY R: Aggression in invertebrates. Curr. Opin. Neurobiol. **13**, 736, **2003**.
20. LAPIED B., GROLLEAU F., SATELLE D. Indoxacarb, an oxadiazine insecticide, blocks insert neuronal sodium channels. Brit. J. Pharmacol. **132**, (2), 587, **2001**.
21. HISSMANN K. Strategies of mate finding in the European field cricket (*Gryllus campestris*) at different population densities: a field study. Ecol. Entomol. **15**, (3), 281, **1990**.
22. SIMMONS L. W., RITCHIE, M. G. Symmetry in the songs of crickets. Proc. R. Soc. Lond. B **263**, 305, **1996**.
23. SIMMONS L. W. Correlates of male quality in the field cricket, *Gryllus campestris* L.: age, size, and symmetry determine pairing success in field populations. Behav. Ecol. **6**, (4), 376, **1994**.
24. HACK M. A. The energetic costs of fighting in the house cricket, *Acheta domesticus* L. Behav. Ecol. **8**, (1), 28, **1995**.
25. NOSIL P. Food fights in house crickets, *Acheta domesticus*, and the effects of body size and hunger level. Can. J. Zool. **80**, (3), 409, **2002**.
26. WANG S.-Y., WANG G. K. Voltage-gated sodium channels as primary targets of diverse lipid-solube neurotoxins. Cell. Signal. **15**, (2), 151, **2003**.