HIBERNATION OF THE PREDATORY STINK BUG *Perillus bioculatus* F. (HEMIPTERA, PENTATOMIDAE) UNDER LABORATORY CONDITIONS

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Abstract. Observations of the laboratory population of predatory bug *Perillus bioculatus* allowed us to establish the time periods and duration of the winter diapause. The imagoes of the 2nd and 3rd generations of the predator proved to be more adapted, while the 4th generation adults of *P. bioculatus* were most vulnerable during hibernation. The mortality rate of *P. bioculatus* individuals in the period from December 15 to March 15 was 12.2; 28.6 and 30.1% for the 2nd, 3rd and 4th generations, respectively. At the same time, the sex ratio (♀ : ♂) was distributed as follows: before the wintering of the 2nd generation – 1:2.9, 3rd – 1:1.3, and 4th – 1:1.4; after coming out of hibernation – for the 2nd generation – 1:2.1, 3rd – 1:1.3 - 1:1.5 and 4th – 1:0.8. It was found that the length of daylight is not the only determining index.

Keywords: predatory stink bug *Perillus bioculatus*, hibernation, overwintering, sex-ratio, generation.

Rezumat. Hibernarea ploşniţei răpitoare *Perillus bioculatus* F. (Hemiptera, Pentatomidae) în condiţii de laborator. Observaţiile asupra populaţiei ploşniţei răpitoare *Perillus bioculatus* au permis stabilirea termenilor şi durata diapauzei de iarnă. În perioada hibernării, adulţii generaţiei a doua şi a treia ale răpitorului *Perillus bioculatus* sunt cei mai adaptaţi, în timp ce, cei din generaţia a patra au fost mai vulnerabili. Mortalitatea indivizilor de *P. bioculatus* în perioada de la 15 decembrie până la 15 martie a constituit 12,2; 28,6 şi 30,1% pentru generaţia a doua, a treia şi a patra, respectiv. Raportul dintre sexe (♀ : ♂) s-a distribuit în felul următor: până la hibernare, pentru generaţia a doua– 1:2,9, a treia– 1:1,3 şi a patra – 1:1,4, după ieşire din hibernare pentru generaţia a doua – 1:2,1, a treia – 1:1,3-1:1,5 şi a patra – 1:0,8. S-a stabilit, că durata luminei zilei nu este unicul factor determinant.

Cuvinte cheie: ploşniţa răpitoare *Perillus bioculatus*, hibernare, iernare, raportul sexelor, generaţie.

INTRODUCTION

Adapting a predator of the Colorado potato beetle (*Leptinotarsa decemlineata* Say, Coleoptera, Chrysomelidae) – *Perillus bioculatus* F. (Hemiptera, Pentatomidae) on the European continent holds great promise for the biological protection of potato against phytophages. Currently, *P. bioculatus* is found in countries such as Bulgaria (SIMOV et al., 2012), Greece (PERICART, 2010), Serbia (PROTIC & NEBOJSA, 2012), Moldova (DERJANSCHI & ELISOVEŢCAIA, 2013, 2014), in the European part of Turkey (KIVAN, 2004; FENT & AKTAC, 2007), as well as in Crimean, Seversk, Slavyansk, Dinsk and Krasnoarmeysk districts of the Krasnodar region, Aksai district of the Rostov region (ARTOHIN et al., 2012), and in the Republic of Adygea (ISMAILOV et al., 2014). Moreover, according to the latest data in 2015, populations of the predator were found in Meerut (UP) North India (PRASAD & RISHI, 2015).

Researchers have gained experience in the study of the predator in laboratory and field conditions and conducted investigations on the selection of artificial diet; the trophic relations of *P. bioculatus*, as well as the influence of abiotic and biotic factors on the population density of the entomophage have been subject to scrutiny. In some countries (Russia, Turkey, Moldova), where the predatory bug adapted to the natural biocoenosis, it was introduced in the laboratory culture and used for mass rearing and release against the Colorado potato beetle (KIVAN & AYSAL, 2014; ISMAILOV et al., 2014; DERJANSCHI & ELISOVEŢCAIA, 2014). However, it is known that a huge role in the preservation of the population is played by the diapause stage when the entomophage is very vulnerable, both in laboratory and in natural conditions. Since the adaptation of the *Perillus bioculatus* species on the European continent took place relatively recently, the predator population is unstable and vulnerable in the new habitat. Maintenance of laboratory culture and mass releases allow to keep the genetic potential of the useful species in the agroecosystems.

Therefore, the aim of our paper was to study the effects of wintering conditions on the numbers and biological indicators of laboratory populations of the predatory bug *Perillus bioculatus*.

MATERIAL AND METHODS

The experiments were carried out during the years 2014-2016 on laboratory population of *P. bioculatus*, introduced into the culture in 2014. The insects were kept in cages, the air humidity was maintained through vessels with salt solution. Nutrition was mixed: *Leptinotarsa decemlineata* and *Galleria mellonella* L. (Lepidoptera: Pyralidae). We took into account the mortality of adult bugs during the preparation for winter diapause, in the torpor period (late autumn - early winter) and after coming out of diapause. We also monitored nutrition, mating, and some other behavioural reactions of *P. bioculatus*.

RESULTS AND DISCUSSIONS

As a result of a laboratory breeding in the conditions of 2014, we prepared for hibernation one population of the predatory bug *Perillus bioculatus* of the 3rd generation. Under the conditions of 2015 there were obtained, for overwintering, already 5 individual populations of the entomophage (three populations of the 2nd generation, and one

population of the 3rd and 4th generation). Under the separate keeping of bugs of different generations it was found that not only adults of the 3rd generation were preparing for hibernation, but also the 2nd (which molted into imagoes in late July - early August). The bugs ate intensely, chose bigger victims (Fig. 1), during the day gathered in large groups, and hid in a shelter, where they spent most of the time, even during daylight hours (Fig. 3). The activity of adults resumed only on sunny days or hours. At the same time, despite the decrease in activity, females of both generations (the 2nd and 3rd) continued to lay eggs. Thus, we had prepared for wintering the fourth generation of the predator – larvae began molting in the imagoes of the 4th generation on September 30 and ended on November 15. The average development duration of the 4th generation larvae, as well as of the 1st, was 24 days (larvae of the 2nd and 3rd generation – 22 and 17 days, respectively). It should be noted that the death of the 4th generation larvae reached 50-80%, despite the abundance of food and constant temperature (+26±1°C) and humidity of 70-75% maintained in the room. Also, in the process of molting, the imagoes of the 4th generation revealed a significant proportion of individuals with defective elytra (Fig. 2) or undeveloped forelimbs - from 10 to 20%. Thus, the duration of the larval stage of the entomophage depended mainly on the temperature and length of daylight, whereas the survival rate of *P. bioculatus* individuals and their biological indicators were greatly influenced, most likely, by the quality of the food provided.

Earlier researchers (STRADIMOVA, 1973) determined that the decisive factor for the survival of the beetle during wintering is its physiological condition. Therefore, for the good preparation of the bugs for the winter diapause, special attention was paid to the quantity and quality of food. The voracity of the adults in late November – early December with decreasing photoperiod decreased slightly, compared to the month of October. It was noted that the bugs selectively treated the offer of food: in abundance of larvae of *Galleria mellonella* (in excess, based on the average daily demand of the bug in feed) the imagoes of *P. bioculatus* ate one caterpillar in groups of 2-5 (Fig. 2). Such behaviour is typical for the bugs, both adults and larvae, throughout the season. However, at the end of autumn, we noted an increase in cases of group nutrition. The ratio of separately feeding individuals and "groups" was distributed in such a way that among 5-6 bugs, and sometimes 7-8, only one fed separately from the group.

A comparative analysis of the bugs' behaviour before hibernation, with naturally decreasing photoperiod, throughout the seasons 2014 and 2015, showed that the decisive role in the formation of diapause of *P. bioculatus* adults was played by factors such as feeding on diapausing hosts, lowering of temperature, as well as the quality and quantity of food. Observations revealed a high mortality rate (till 51.7%) among *P. bioculatus* imagoes of the 4th generation in the last months of autumn (late October) and prior to their dormancy (the first decade of December). Among the imagoes of the 2nd and 3rd generations in the year 2014-2015 the mortality rate was significantly lower – 12.1-28.8% (Table 1). The period from August 9, 2014 to September 17, 2015 was conditionally accepted by us as settlement dates for the calculation of imago deaths before hibernation, as at this time the composition of populations of the 2nd and 3rd generations are no longer replenished with new individuals.

Table 1. Mortality rates in the laboratory populations of *Perillus bioculatus* in hibernation.

Generation of the *Perillus bioculatus*	**Percent mortalities (%)**			**Survival rate of adults (%)**
	Before hibernation	During hibernation	Total	
Year (period)	2014-2015			
Data (period)	September 9, 2014 – December 14, 2014	December 14, 2014 – March 31, 2015	September 17, 2014 – March 31, 2015	March 31, 2015
3rd	18.2	22.2	36.4	77.8
Year (period)	2015-2016			
Data (period)	September 17, 2015 – December 10, 2015	December 10, 2015 – March 15, 2016	September 17, 2015 – March 15, 2016	March 15, 2016
2nd	12.1	12.2	27.3	87.8
3rd	28.8	28.6	50.0	71.4
4th	51.7	30.1	75.9	69.9
		$HSD_{0.05}$=6.4		$HSD_{0.05}$=6.4

As it can be seen from Table 1, the most adapted to wintering were the second generation imagoes, their death rate during the period of hibernation (December 10, 2015 – March 15, 2016) being only 12.2%, while for the third and fourth generations it reached 28.6 and 30.1%. It was established that the duration of the winter diapause of *P. bioculatus* in 2014 was 3.5 months, and about 3 months in the conditions of 2015. The air temperature in the winter months and in the first half of March 2015 was higher than in 2014. This explains the different dates of waking up from hibernation of the imagoes. The bugs emerged when the average daily air temperature reached +12°C. Once the cages were transferred into a warm room, the imagoes proceeded to mating, feeding only after 2-3 days (Fig. 4).

As a result of the analysis of the data, it was determined that at the time the populations woke up from hibernation, the ratio of survivors ranged from 69.9 to 87.8%, depending on the generation (Table 1). The mathematical analysis of the data revealed a significant difference ($HSD_{0.05}$=6.4, χ= from 10.0 to 17.9) between the viability of adult *P. bioculatus* of the 2nd, 3rd and 4th generations during winter. At the same time, between the viability of the 3rd generation imagoes during the wintering period 2014-2015, there was not observed a significant difference ($HSD_{0.05}$=6.4, χ= 6.4).

We also determined the sex ratio of *P. bioculatus* adults before and after hibernation (Table 2).

Table 2. Sex ratio in the laboratory populations of *Perillus bioculatus* in hibernation.

Generation of the *Perillus bioculatus*	**Sex ratio (♀:♂)**	
	Before hibernation	**After hibernation**
Year (period)	2014-2015	
3rd	1:1.3	1:1.3
Year (period)	2015-2016	
2nd	1:2.9	1:2.1
3rd	1:1.3	1:1.5
4th	1:1.4	1:0.8

Figure 1. Imago of *P. bioculatus*, eating a larvae of *Galleria mellonella* before hibernation (original).

Figure 2. Imagoes of the 4th generation *P. bioculatus* – defective development of the elytrum on the right down (original).

Figure 3. Aggregation of the imagoes of *P. bioculatus* during hibernation (original).

Figure 4. Coupling of the imagoes of *P. bioculatus* on the third day after hibernation (original).

After the molting of *P. bioculatus* larvae into imago, the sex ratio is approximately 1:1, with minor deviations. However, later in the laboratory breeding, the sex ratio often varied towards male dominance. During hibernation and in the first two weeks after hibernation, the mortality rate among males is on an average higher than among females. Clearly, the viability of diapausing females is higher than that of males, which explains the cause of male dominance in a quantitative sense before hibernation.

CONCLUSIONS

Our data on the survival rate of adults of predatory bug *P. bioculatus* of the second – fourth generations during winter diapause allows to take into account factors that significantly affect the formation of diapause, as well as the viability of the species. The targeted selection of the best prepared for wintering populations in the conditions of laboratory breeding will help to optimize the process and reduce direct costs.

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Received: March 23, 2016
Accepted: April 26, 2016