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NATURAL REPRODUCTION OF ENTOMOPHAGES TO RESTORE BIOCENOTIC REGULATION IN CEREAL CROPS

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Abstract

Recently, plant protection methods based on pest regulation by entomophages, naturally inhabiting the crops or attracted thereto, become more preferable. This relevant approach to agro landscape construction necessitates seeking for ways to activate natural cenotic mechanisms, e.g. via optimized crop rotation, created micro reservoirs of desirable species, botanical diversity of protective forest belts, roadsides and other natural plant communities etc. Telenomines (*Hymenoptera: Platygastroidae: Scelionida, Telenominae* subfamily), the egg parasites of *E. integriceps*, are a powerful regulator of sunn pest *Eurygaster integriceps* Put. abundance. These entomophages are of particular interest worldwide as they are the most numerous not only on bread wheats but also on tilled crops. For a long time, researchers from Russia and other wheat producing countries, where *E. integriceps* pests pose a threat to crops, were concentrated on the laboratory reproduction of telenomines for field use. However, exploitation of naturally reproduced telenomines has not yet been studied. This paper is the first to report on use of entomophilic fennel strip plots with arranged kairomone-based feeding places as baits for *Graphosoma lineatum* L., the alternative hosts of telenomines, to further attract and massively reproduce these egg parasites of *E. integriceps* in field conditions. This technology is based on a long time study (2006-2018) of factors affecting reproduction of telenomines in cereal cenoses. Our data indicate the key role of winter generation of these egg parasites, which reproduce successfully during the second half of the season last year, in the harmful bug control. Additional hosts, the bugs of *Pentatomidae* family among which the Italian striped bug *G. lineatum* is the most frequent (7.20±1.20 insects per square meter), provide a significant increase in count of wintering telenomines. Sunflower and soybean agrocenoses with bait strip plots of entomophilic fennel (coriander) are optimal to provide reproduction of telenomines. In the kairomone-based feeding places, telenomines laid their eggs into 52.1±2.2 to 84.9±3.2 % eggs of *G. lineatum.* Due to use of this technology during 2008-2010, farther use of kairomone-based feeding places for accumulation of telenomines was not needed. Currently, the entomophages actively reproduce due to crop rotations with up to 40 % cultivated plants favorable for *Scelionidae* species. In experimental wheat crops of All-Russian Research Institute of Biological Plant Protection, in 2011-2017 from 53.1±2.7 до 88.2±2.0 % eggs of *E. integriceps* contained eggs of telenomines. This allowed for cancellation of other protective measures against the bug's larvae.

Keywords: phytophagous insects, *Eurygaster integriceps* Put., sunn pest, *Graphosoma lineatum* L., shield bugs, entomophages, egg parasites, telenomines, reproduction, natural habitats, spatial patterns, grain crops, tilled crops, kairomone-based feeding places, biocenotic regulation, efficiency

Ecologically oriented agriculture involves optimization of phytosanitary measures based on the predominant substitution of synthetic plant protection products [1]. Avoiding chemical pesticides provides the maximum use of self-regulation mechanisms in cenoses to reduce the number of pests [2-4]. Natural aboriginal entomophages are one of the biotic factors determining the stability of biocenotic regulation of phytophage populations in the biological protection system [5-9].

Wheat is the leading cereal in many countries, including Russia. Winter wheat crops occupy more than 30% of arable land (up to 1.6 million hectares) annually in the south-west of the North Caucasus in the conditions of the Krasnodar Territory. There are up to 30-40 species of phytophages in the agrocenosis of the wheat field in the Kuban region, including 18 specialized ones, to 7-8 species of which chemical treatments are applied annually [3, 7-9]. The most dangerous threat is the corn bug (*Eurygaster integriceps* Put.) that reduces the grain quality; the greatest amount of pesticides accounts for the treatments against this pest [10-13]. The corn bug has a wide geographical range: it is common in the steppe zone in the south of the forest-steppe of Russia and Ukraine, in the Caucasus, in Albania, Greece, Bulgaria, Romania, Turkey, Syria, Lebanon, Iran, Iraq, Afghanistan, and Pakistan [14].

The most effective entomophages of *E. integriceps* Put. include egg-eating parasites from the *Scelionidae* family [3, 4, 7, 8, 15]. In the general geographical range of the bug, including the areas close to the Russian Caucasus in the south, e.g. in Turkey, Iran, Iraq [16-20], as well as in the region of wheat cultivation located to the north, in the Volga Region [21], about 20 species of telenomines are described, mainly from the *Trissolcus* and *Telenomus* genera. In some years, they are able to infect up to 80% of the pest egg-laying, but the mechanisms regulating the increase in activity and the natural reproduction of this group of parasites are not well studied [7, 8, 22]. In Russia and other countries, technologies for mass cultivation of telenomines under laboratory conditions have been developed for many decades, both using host insects, mainly on the striped bug *Graphosoma lineatum* L., and using artificial nutrient media (ANM) [23-25]. However, this method is labor-consuming and expensive, since entomophages develop on ANM for 3-5 days longer than on the insect host [23].

In the USSR, as early as 1940-1975, on the basis of a long-term analysis of seasonal colonization of local and introduced species of the egg-eaters of the *Trissolcus* genus (*Tr. grandis* Thoms., *Tr. simoni* Mayr and others), attempts were undertaken to introduce large-scale practical use of these entomophages for controlling phytophages, which, however, did not give a positive result [25]. The authors' long-term studies [7, 26] showed that the cause was the death of laboratory populations of *Trissolcus* in early spring due to their inherent increased xero-and thermophilicity.

An essential role in maintaining the potential of the telenomines of the *Scelionidae* family is played by their additional hosts, the stink bugs of the *Pentatomidae* family, which ensure the survival of telenomines in natural conditions before and after the eggs are laid by the corn bug [6, 7, 19]. According to different sources, these are mainly representatives of *Hemiptera* family, the *Pentatomidae* (20-30 species). The known domestic and foreign publications indicate only the species composition of these *Hemiptera* groups whereas their role in ensuring the maximum effectiveness of telenomines remains insufficiently studied [26-29].

This study for the first time determines that the key role in ensuring the reproduction of overwintering stock of egg-eating telenomines, which are the main regulators of the corn bug abundance in the North Caucasus, is played by their additional hosts, bugs of the *Pentatomidae* family, which are constantly present in the agrocenosis of agricultural crops.

The purpose of the paper was to identify the factors that determine the increase in the activity and reproduction of egg-eating telenomines in the cenosis of grain crops, taking into account the features of the stationary distribution and the reproduction rate of their additional hosts, the *Pentatomidae* bugs.

Techniques. Observations (2006-2018) were carried out with respect to the most common species of stink bugs, the sloe bug (*Dolycoris baccarum* L.), the striped bug (*G. lineatum* L.), the pointed head bug *Aelia acuminata* L., *Car-*

pocoris fuscispinus Boh. and rape bug (*Eurydema oleracea* L.), which ensure the preservation and accumulation of the aboriginal populations of the corn bug (*E. integriceps* Put.) egg parasites, as well as the dominant species of the corn bug egg-eating telenomines, the *Telenomus chloropus* Thoms., *Tr. grandis* and *Tr. simoni* regulating the number of pests. Surveyed plots were located in the Central zone of the Krasnodar Territory (experimental crop rotation of the All-Russian Research Institute of Biological Plant Protection, RIBPP, a total area of 247 hectares, 2006-2017); OAO Chistaya yeda (Krymsky District, Krasnodar Territory, 200 hectares, 2011-2013); farms of the south of the Rostov Province (300 hectares, 2014-2015). The soil type of the Central zone of the Krasnodar Territory is southern leached chernozem, the weather conditions during the years of observation were mostly typical for the region: the average monthly temperature was 21.7 °C, the air humidity was 66.9% with the warm humid weather in July (the hydrothermal coefficient (HTC) was 0.9-2.2), dry hot weather in August (HTC 0.01-0.10) with severe drought in July-August (HTC 0.04-0.20) in 2007 and 2010. The zone is characterized by the presence of a network of field-protective belts. The southern part of the Rostov region belongs to the steppe zone, the soil is ordinary black chernozem, weather conditions typical for the region: the average monthly temperature is 19.6 °C, the air humidity is 69.9%.

In field experiments, cages-insulators (aluminum frame 20-25 cm in diameter and 13-15 cm in height with stretched mesh material, which allows free access of telenomines) were used as kairomone feeding pads (KFP). Insulators were secured at the top with rubber rings for limiting the movement of the bugs. One drinking trough with a moistened cotton swab and feeders with dill seeds or wheat germ were placed at the bottom of each KFP. The number, species composition and reservation sites of the corn bug egg parasites were determined using KFP. Males and females of *G. lineatum* striped bug and *E. integriceps* corn bug from natural populations were used as a live source of kairomone and ovipositors. *G. lineatum* specimens were selected on special small (up to 50 m^2) plots of dill (*Anethum graveolens* L.) or coriander (*Coriandrum sativum* L.), and the corn bug was selected on wheat crops.

To account the field number of adults and bugs larvae, their species composition and reservation sites, mowing was carried out using a standard entomological net with a diameter of 30 cm [30]. The infestation of bug eggs with telenomines and the species composition of the latter were studied during individual and mass laboratory breeding of parasites using phytophage ovipositors collected in the field [30]. The concentration sites, the species composition and the number of egg parasites of the corn bug were determined using KFP, which ensured the attraction of telenomines.

The taxonomic identity of telenomines was identified by the determinant of the Zoological Institute of the Russian Academy of Sciences (St. Petersburg) [31] by viewing under an MBS-10 binocular microscope (magnification ×8, OAO LZOS, Russia). The insects were kept in MLR 35 OH artificial climate chambers (Panasonic, Japan).

The number of stink bugs of the *Pentatomidae* family was counted monthly. The obtained results were processed according to generally accepted methods of statistical analysis using the Statistica v.12.6 software (StatSoft, Inc., USA) [32]. The tables show the mean (*M*) and standard error of the mean (±SEM).

Results. To attract entomophages of the corn bug, kairomones were used, which are considered as effective agents against this pest [33]. In field experiments to assess the natural distribution, as well as for attraction and reproduction of telenomines, specially designed kairomone feeding pads (KFP) were used.

Kairomone feeding pad used in the experiment for recording, attracting and reproducing egg-eating telenomines of the corn bug: 1 — cage-insulator, 2 — drinking trough with a moistened cotton swab, 3 — feeder with dill seeds.

Long-term landscape biotopical monitoring of the stink bugs on winter wheat crops (2006-2017) showed that bioecologies of the *Pentatomidae* family representatives are generally similar. Insects overwinter in the imago stage under the canopy of trees and shrubs, mainly oak (*Quercus robur* L.), as well as on the fields and roadsides under the remains of grassy plants. At an average daily temperature above 11 °C, the bugs came out of the overwintering areas almost simultaneously with the telenomines (1 month before egg-laying by the corn bug). An exception was the striped shield bug (*G. lineatum*) which left the overwintering at an average daily temperature above 20 °C simultaneously with *E. integriceps*. Development cycles of the corn bug and the striped shield bug coincide, but shield bugs do not live on wheat. After completing feeding on young leaves of oak, acacia (*Caragana arborescens* L.), ash (*Fraxinus excelsior* L.), and blackthorn (*Prunus spinosa* L.), shield bugs mated.

1. Number (ind/m^2) **of *Pentatomidae* family shield bugs in the studied agrobiocenoses** (M±SEM, RIBPP, Krasnodar, 2006-2017)

Month	*Carpocoris fuscispinus* Boh.	*Dolycoris baccarum* L.	*Aelia acuminata* L.	*Graphosoma lineatum* L.	*Eurydema oleracea* L
Flowering herbs					
April	1.60±0.30	4.04±1.10	0.09±0.09	0.20±0.08	0.70±0.09
May	1.10±0.50	1.50±0.80	0.90±0.10	0.40±0.03	0.20±0.07
June	0.70±0.40	3.03±1.10	0.40±0.25	0.50±0.07	0.02±0.10
July	–	–	–	0.70±0.16	0.70±0.09
Winter rape					
April	0.02±0.10	0.70±0.15	0.20±0.09	–	1.07±0.70
May	0.10±0.07	0.20±0.10	0.10±0.09	–	1.03±0.65
Winter wheat					
April	0.10±0.08	0.20±0.10	0.30±0.10	–	0.10±0.07
May	0.30±0.11	1.00±0.15	1.20±0.20	–	0.10±0.07
June	0.20±0.09	0.90±0.17	1.50±0.20	–	–
Corn					
July	0.10±0.06	0.80±0.10	–	–	–
Sunflower					
July	0.10±0.08	0.50±0.09	–	–	–
August	–	0.10±0.08	–	–	–
Soy					
July	0.10±0.07	0.06±0.23	–	–	–
Август	0.01±0.08	0.30±0.11	–	–	–
Entomophilous cultures (dill, coriander)					
May	–	–	–	0.60±0.11	–
June	–	0.30±0.11	–	3.30±0.75	–
July	0.10±0.06	0.30±0.12	–	7.20±1.20	–

Note. A dash means that no shield bugs were found on the plants.

During the formation of reproductive products (usually the 2nd decade of April), the bugs in the forest shelter belts were inhabiting wild plants from different botanical families (*Asteraceae*, *Lamiaceae*, *Caryophyllaceae*, *Brassicaceae* and *Poaceae*) eating their generative organs. In these areas, the number of shield bugs was many times greater than that of the cultural station, i.e. in crops of early flowering winter rape (Table 1). Under favorable weather conditions, at the end of April, most of the shield bugs began egg-laying, which passed into the mass egg-laying in the winter wheat cenosis. Striped shield bugs developed on flowering umbrella plants, rape bug developed on winter rape (the number of *E. oleracea* during all the years of observation was small, therefore infestation of its egg-laying was not determined).

Due to the earlier egg-laying of the shield bugs, which begins 7-10 days earlier than that of the corn bug, there was an increase in the number of egg parasites (5 times or more) and in occupation by their first generation of the wheat crops adjacent to the field-protective plantations, which allowed overwintering telenomines populations to wait for the *E. integriceps* eggs. Thus, asynchrony in the development of parasites and their host was overcome to some extent, which increased the efficiency of *Scelionidae* [7, 26].

Colonization of the winter wheat by the shield bugs began during the stem elongation phase (Z 31-35 by Zadoks scale). Biocenosis of wheat in the Krasnodar Territory is characterized by the 6 species of pentatomids, among which the most common are the pointed head bug (*A. acuminata*), the sloe bug (*D. baccarum*) and *C. fuscispinus*, while the pointed head bug occupied the wheat before the other shield bugs (7 days earlier) and finished the egg-laying later (14 days later) [26]. The development cycles and the nature of harmfulness of the *D. baccarum* and *C. fuscispinus* almost coincide. Shield bugs cause white-spikes and underdeveloped ears, and later the change in physiological properties of the grain [34, 35].

2. Infestation of corn bug (*Eurydema integriceps* Put.) eggs and shield bugs of the *Pentatomidae* family with telenomines of the *Trissolcus* genus on winter wheat crops (*M*±SEM, RIBPP, Krasnodar, 2007-2013)

Wheat development phase (according to Zadoks)	Analyzed eggs, pcs	Infested by parasites	
		total	%
Corn bug			
Z 31-35	140.0±6.7	99.0±2.5	70.7±4.4
Z 50-59	610.0±5.5	521.0±7.5	85.4±3.2
Z 70-75	590.0±4.8	557.0±3.3	94.4±3.6
Z 75-80	260.0±3.2	244.0±5.0	93.8±5.1
Shield bugs (during egg laying by corn bug)			
Z 31-35	70.0±2.6	25.0±1.7	35.7±4.4
Z 50-59	70.0±4.1	28.0±2.2	40.2±2.4
Z 70-75	90.0±3.9	41.0±1.5	45.6±3.7
Z 75-80	250.0±3.1	201.0±7.2	80.4±3.6
Shield bugs (after completing egg laying by corn bug)			
Z 75-80	510.0±6.7	470.0±5.9	92.1±4.8
Z 80-90	710.0±7.8	610.0±6.3	85.9±4.1

Note. Infestation rate is an indicator of EEL (entomophage efficiency level).

The number of *Pentatomidae* bugs in the cenosis of winter wheat during observations did not reach the economic threshold of harmfulness (3.0-3.5 ind/m^2) due to the activity of their natural enemies, the egg-eating *Scelionidae* wasps. Parasites infested up to 80.4±3.6% of shield bugs eggs and 93.8±5.1% of *E. Integriceps* eggs (Table 2). The first egg-laying of both the shield bugs and the corn bug were 80-100% affected by hygrophilic species *T. chloropus* Thorns. After completing of the egg-laying by the corn bug, development of the third generation of egg-eaters (phases of wax and full ripeness of grain, Z 75-90) continued on the eggs of additional hosts, the shield bugs of *Dolycoris*, *Aelia*, *Carpocoris* genera. The infestation rate of their eggs by telenomines in the absence of egg-laying of the main host reached 92.1±4.8%. Such values were recorded during almost the entire observation period (see Table 2).

An important condition for the development of telenomines is the presence of eggs of their additional hosts in other stations [24, 28, 29]. When noting this fact, the authors, however, did not determine which conditions contributed to the preservation of entomophages, in essence, limiting themselves to analyzing the taxonomic structure of the described groups of insects. Only a few papers report on the infestation of up to 30% of eggs in *Dolycoris* genus [18, 28]. The authors of this paper showed that at the end of the grain crops harvesting, the shield bugs migrated to row crops and plantings with a variety of flowering grassy vegetation in search of food. Sunflower and soybeans during budding—flowering were the most attractive for pentatomids in the indicated timeframes, crops of entomophilous dill (coriander) and, to a lesser extent, melliferous umbrella plants for the shield bugs. The formation of the telenomines overwintering stock is most intense in the biocenoses of these cultures. On average, the number of striped shield bug eggs per KFP ranged from 1170.0±6.5 to 2460.0±8.3, among them 610.0±4.4 to 2090±7.3 were attacked by egg parasites. Greater infestation of egg-laying by telenomines was noted near the crops of entomophilous dill, 2090±7.3 of 2460±8.3 individuals (84.9±3.2%).

3. Infestation of shield bug eggs (*Graphosoma lineatum* L.) by telenomines of the *Trissolcus* genus on the kairomone feeding pads, providing optimal conditions for attracting and reproducing the telenomines (*M*±SEM, RIBPP, Krasnodar, 2006-2010)

Crop	Laid eggs	Infected by telenomines	
		total	%
Sunflower	2440.0±7.2	140.0±3.0	57.4±3.0
Corn	1170.0±6.5	610.0±4.4	52.1±2.2
Soy	1790.0±7.9	1080.0±6.9	60.3±4.2
Dill, coriander	2460.0±8.3	2090.0±7.3	85.0±3.2

Note. Infestation rate is an indicator of EEL (entomophage efficiency level).

Reactivation of egg-eaters and their spread to the fields in early spring occurred at a lower temperature (11-12 °C) than the beginning of the egg-laying of the corn bug (20 °C), which led to the death of a significant part of overwintered egg parasites [6, 26]. According to this study, telenomines survival is facilitated by the presence of flowering plants in their habitats during this period (insects eat their nectar), so the highest infection rate of the shield bugs eggs by telenomines on KFP was noted in wheat crops near the flowering wild-growing grass [3, 7, 26]. Therefore, it is very important to keep intact the borders of the forest belts with grassy vegetation as places for wintering, feeding and breeding entomophages and those phytophages that may be used as additional hosts of entomophages [3, 6, 7, 36].

According to the authors' data, the number of *Scelionidae* on cereal crops can be increased by creating their additional reserves when grown in crop rotations of entomophilous dill for the accumulation of the striped shield bugs. To this end, in 2008-2010 the method of mass reproduction of the egg-parasites of the corn bug at the entomophilous dill sites in the field was tested. The originality of the approach used is that synchronous reproduction of egg-eaters and their additional host, *G. lineatum*, occurred on dill. The proposed technology was as follows. The strips of planted entomophilous dill, on which, during the development of the striped shield bug summer generation (July—August) the KFP was installed in a row with 10 m spacing (a KFP per 4 hectares of protected wheat crops), were placed near areas of overwintering and constant reproduction of telenomines and their additional host, namely, near the trees and shrubs, orchards, row-crops plantings (corn, soybean, sunflower). Mass accumulation of telenomines in natural cenoses included i) the production of the insect host, a polyvoltine striped shield bug, by growing entomophilic dill (or coriander), which ensures attraction and accumulation of the bug; ii) mass reproduction of entomophage parasites in KFPs located in the agrocenoses of tilled crops (maize, soybean, sunflower) and in bait plots of dill (coriander) (to preserve and increase the overwintering stock of entomophages); iii) cultivation of nectariferous crops (phacelia, coriander, fennel) for additional feeding of egg-eaters; iv) testing the ability of breeding parasites to control the pest (the corn bug). It should be noted that that the previous studies [5, 8, 22, 27, 28] do not show the possibility of breeding *Scelionidae* by providing field conditions conducive to reproduction and preservation of their additional hosts. A number of papers propose mass production of entomophages grown in the laboratory, but their efficiency often did not exceed 50% [6, 24].

The intensive use of the developed technology during 2008-2010 allowed abandoning the KFPs for additional accumulation of telenomines. The reproduction of entomophages is carried out by maintaining 7-40% of tilled, entomophilous and nectar-bearing crops in the structure of crop rotation, due to which the populations of aboriginal species of the *Scelionidae* family are activated. During the last 9 years, telenomines infested from 53.1±2.7 to 88.2±2.0% of the corn bug eggs in the experimental crop rotation (All-Russian Research Institute of Biological Plant Protection), which allows excluding all treatments with preparations against the bug larvae in the grain milky phase (such treatments are recommended in case of egg-laying infection below 40-50%) (Table 4). The number of bug larvae born of uninfected eggs did not exceed 2.1 ind/m^2 on average.

4. Prolonged effect of crop biodiversity in crop rotation while maintaining the natural reproduction of *Scelionidae* family telenomines populations on winter wheat crops (*M*±SEM, kairomone feeding pads, RIBPP, Krasnodar, 2010-2018)

Year	Ovipositor of corn bug (*Eurygaster integriceps* Put.)		
	eggs laid in KFP	eggs infested by telenomines	
		total	%
2010	280.0±4.1	210.0±3.2	75.0±1.8
2011	380.0±4.2	240.0±2.6	63.1±3.3
2012	340.0±5.1	210.0±3.8	61.7±4.0
2013	360.0±5.0	290.0±6.1	80.5±2.2
2014	510.0±3.1	450.0±4.4	88.2±2.0
2015	490.0±4.6	260.0±5.1	53.1±2.7
2016	560.0±4.1	360.0±4.3	64.2±1.9
2017	550.0±2.9	420.0±3.8	76.3±3.1
2018	520.0±4.5	405.0±1.9	77.8±2.6

N o t e. Infestation rate is an indicator of EEL (entomophage efficiency level).

Elements of the technology (crop rotations with sunflower and soybean, strip crops of entomophilous dill) in 2011-2013 passed production testing in the Krasnodar Territory (OAO Chistaya yeda, Krymsky District) and in 2014-2015 in the Rostov region (KFH Biokhutor, Taganrogsky District, SPK AF Novobatayskaya, Kagalnitsky District). After 2-3 years of using the proposed techniques, the infection rate of *E. integriceps* egg-laying with telenomines was 45.5-69.0% regardless of weather conditions. In each farm, the area of chemical treatments against the pest was reduced by 500 hectares on average. At the same time, the number of its imago and larvae remained below the economic threshold of harmfulness (no more than 1.8 ind/m^2).

Thus, this paper has shown that the preservation and reproduction of the main egg-eating telenomines with the participation of their additional hosts, the shield bugs, under natural conditions is provided by the inclusion of tilled and entomophilous crops in the crop rotation. This for the first time made it possible to form, and subsequently, by constantly sowing tilled, entomophilous and nectariferous crops in crop rotation, to maintain the "natural biolaboratory" in agroecosystems for restoring the natural biocentric regulation of phytophage numbers by entomophages in wheat crops. In such natural biolaboratory, the wintering stock of telenomines is activated and reproduced during the second half of the summer season. The proposed technology for 9 years provides 88.2% infestation of the corn bug laid eggs by egg-eaters of *Scelionidae* family, which made it possible to completely abandon chemical treatments against the pest.

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