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BIOCONTROL OF AGRICULTURAL PESTS BASED ON AUTODISSEMINATION OF ENTOMOPATHOGENIC NEMATODES OF *Steinermatidae* FAMILY (*Nematoda: Rhabditida*)

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Abstract

At present, the development of biological plant protection systems is among the most important economic, social and environmental challenges. Creating an effective system is impossible without the combination of a variety of biological agents and pest control techniques. Combining a variety of pathogenic organisms and synthetic sex pheromones is a way to improve the situation. This paper shows the effectiveness of entomopathogenic nematodes (EPN) of the family *Steinernematidae* Filipjev, 1934 as an autodissemination agent for agro-ecosystems under crop rotation and at apple-tree orchard, and assessed the effect of introducing pathogens on indigenous entomopathogens in soil. In particular, a decrease in the number of harmful insects and an increase in the activity of natural beneficial entomopathogens have been demonstrated. The essence of the method consists in the targeted introduction of entomopathogenic bioagents into the agro-ecocenosis by means of their application to attracted insects caught in traps, and thus creating an epizootic in the populations of target species. Previously, entomopathogenic nematodes were not used as autodissemination agents against superdominant species, the codling moth *Cydia pomonella* L., 1758 and click beetles of the family *Elateridae* Leach, 1815; moreover, their effect on other members of the entomofauna of agro-ecocenoses has not been studied either. The purpose of this work was to evaluate the effectiveness of the EPN autodissemination method for various cultures. The successful testing of Granulosis virus dissemination method in the apple orchard and the EPN autodissemination against wireworms prompted us to conduct the investigation reported herein. Two species of entomopathogenic nematodes of *Steinernematidae* family, the *Steinernema carpocapsae* (Weiser, 1955) and *St. feltiae* (Filipiev, 1934) were reproduced in lab culture in different host insects to produce nematode inoculums. The experiments found out that specially designed formulations and modified pheromone traps ensure EPN introduction into the agrocenoses due to nematode invasion of trapped insects followed by their free flight to spread pathogens. As a result, the nematode-bacteria complex occurred in 60.0-100 % of click beetles of the *Elateridae* family and 34.0-35.3 % of *C. pomonella* L. and *Grapholitha molesta* (Busck, 1916). This indicates accumulation of biocontrol agents in the soil of the agrocenoses due to EPN introduction. The EPN autodissemination application also reduced the damage to apple fruits by up to 10 %, and corn and soybean plants by 13,2 % compared to areas where chemical treatments were applied. The method has no negative impact of EPN on green lacewings (*Chrysopidae* Schneider, 1851) and the *Hymenoptera* of the families *Braconidae*, Latreille, 1829 and *Ichneumonidae*, Latreille, 1802, the predators of insect pests. In the garden where the tests were carried out, there was a 15 % increase in infection of caterpillars of *C. pomonella* by *Hymenoptera*. It is established that the EPN autodissemination stimulates the activity of indigenous soil EPN, leading to a 1.5-2.0-fold increase in the number of trapped nematodes in the bioassay test compared to the period prior to EPN autodissemination. Importantly, the effect of autodissemination turned out to be prolonged and manifested the next year both in the apple orchard and in the crop rotation of

agricultural crops.

Keywords: biocontrol, entomopathogenic nematodes, autodissemination, soil nematodes, codling moth, click beetles, wireworms, pheromone traps, apple trees, maize, soybean

The excessive use of chemical pesticides in Russia raises concerns about the safety of products and the environment, therefore, natural regulators of biocenotic relations in agricultural systems is becoming a new strategy for protecting plants from harmful organisms [1].

Use of synthetic insect sex pheromones is a technology of pest population biocontrol on the most important agricultural crops [1-5]. These attractants used worldwide for monitoring, mass capture and disturbance of reproductive connections (disorientation) of phytophages have not yet exhausted themselves [6-11]. For many insect species in the agrocenosis, it was found that with an increase in the concentration of synthetic sex pheromone, egg laying decreases, time for development of preimaginal stages lengthens and their survival decreases, which, in particular, has been demonstrated in the garden for apple and eastern moth [4, 12 -15]. The search for new methods of using insect sex pheromones to control pest population led to the discovery of original methods based on the dissemination of pheromones and autodissemination of entomopathogens of target species using pheromone traps with applicating devices [15, 16].

Entomopathogenic nematodes (EPN) of the families *Steinernematidae* Filipjev, 1934 and *Heterorhabditidae* Poinar, 1976 are effective natural bioagents. These organisms can infect more than a thousand species of arthropods, many of which are dangerous pests of the most important agricultural crops [17-20). Among biological agents produced in the world, nematode preparations are in second place after bacterial ones [21-25]. EPNs have the ability to independently penetrate into the prey, survive in dead insects and contribute to the invasion of other pathogens (in particular, viruses and bacteria) of entomopathogenic parasites into the insect body. The high development rate of nematodes (in the body of a living host for 6-10 days) allows them to spread with larvae and imago of pests [26-31]). The only factor limiting the widespread use of EPN-based drugs is their high hygrophilicity. Therefore, to increase the viability of these bioagents, various formulations are developed that provide sufficient and prolonged moisture supply to nematodes [17, 23-26]. It is also important that these pathogens, interacting with other consorbents of the biocenosis, can also play a microregulatory role in the formation of the soil structure [32, 33]. The peculiarities of the EPN biology suggest the possibility of saturating the soils of agrocenoses with them using males caught with pheromone traps with applicators which infect attracted insects with entomopathogenic nematodes (the method of autodissemination of entomopathogens). Many studies have shown a high biological effectiveness of EPN against wireworms (larvae of click beetles of the family *Elateridae*) and the codling moth *Cydia pomonella* L., 1758, and various ways of their use were considered [23, 34-36].

Here, we propose a new method for application of entomopathogenic nematodes by autodissemination which ensures their long life span, high activity and speedy propagation. The method is based on intra- and interspecific chemical communication and positive phototaxis of insect species attracted by synthetic sex pheromones or light into the applicating devices [16]. Insects leaving the applicator act as EPN carriers used to protect agricultural crops from dominant pests [16, 35].

Our goal was to assess whether pheromone traps can be used for autodissemination of entomopathogenic nematodes as biocontrol agents for the fruit moth *Cydia pomonella* L., 1758 and click beetles from the family *Elateridae* Leach, 1815,

and how they affect the beneficial fauna in agrocenoses.

Materials and methods. Three species and four variants of entomopathogenic nematodes (*Steinernematidae* family), the *Steinernema feltiae* (Filipiev, 1934), *St. kraussei* Steiner, 1923, *St. carpocapsae* (Weiser, 1955), *St. carpocapsae* var. "*agriotes*" (Weiser, 1955) from the collection of useful organisms of the Federal Scientific Center for Biological Plant Protection (FSCBPP) were used. At first *St. kraussei* was derived from the collection of the All-Russian Research Institute of Fundamental and Applied Parasitology of Animals and Plants RAS (VNIIP, Moscow), *St. feltiae* and *St. carpocapsae* var. "*agriotes*" were obtained from the collection of the All-Russian Research Institute of Plant Protection (VIZR, St. Petersburg—Pushkin). *St. carpocapsae* is a local form found in an apple orchard in the Leningradskaya village (Krasnodar Territory).

The ESP was propagated in the laboratory insect hosts greater wax moth *Galleria melonella* (Linnaeus, 1758) and yellow mealworm beetle *Tenebrio molitor* Linnaeus, 1758 (an MLR 35 OH artificial climate chamber, Panasonic, Japan) in as per Danilov's method [24] with our modifications [35, 36].

The detection of entomopathogenic nematodes in soil samples from the biotopes was carried out according to Spiridonov [27], using 10 caterpillars of *G. melonella* placed in each soil sample taken at control sites and the sites of autodessemination. After 1 week, the caterpillars were removed and the entomopathogens were detected (a microscope Biolam, LOMO, Russia, 90× magnification); soil samples were also examined under a binocular microscope at 10× magnification (MBS-10, LZOS, Russia) [27].

After autodissemination, entomopathogens were detected in adults, larvae, and caterpillars using the so-called "nematode traps" and by viewing the biomaterial under a microscope (Biolam, LOMO, Russia, 90× magnification).

To infest three species of click beetles, the Kuban *Agriotes tauricus* Heyden, 1882, common click beetle *A. sputator* (Linnaeus, 1758), and steppe wireworm *A. gurgistanus* (Faldermann, 1835), modified standard Estron-type traps (manufactured by the All-Russian Plant Quarantine Center, Moscow Province) were used. A 45×95 mm foam rubber sponge impregnated with an EPN suspension with the titer of invasive larvae 2.5×10^6/ml was put inside. To ensure free migration of captured insects into the environment, a 35 mm flight hole was made in the insect receiver. Ten traps per each treatment were set depending on the beginning of flight period of each studied click beetle species, distributed evenly over the soybean and corn plots at a distance of 30-40 m from each other according to the "envelope" scheme. For proper isolation, the test sites were located at a 200 m distance. A freshly prepared suspension of nematodes was added to sponges and sampling was performed every 7-10 days during the entire flight period of insects. To count the captured male click beetles, half of the traps were without an air hole. The number of the infected beetles and invasive larvae of entomopathogenic nematodes released from them were determined in lab tests.

For dissemination of EPN in the apple orchard, we used standard modified traps of the Atracon-A type (made by us from Tetrapak paper), 10 traps per treatment. To apply nematodes, a 20×20 mm foam rubber sponge with a suspension of entonematodes (a titer of invasive larvae of 2.5×10^6/ml) was placed inside. To determine the number of caterpillars infected with nematodes, we used traps with glue inserts; for the further spread of EPN in the agrocenosis, half of the traps did not contain glue inserts.

Every 7-10 day, a freshly prepared suspension of nematodes was applied

to the sponges and sampling was carried out (the frequency of changing the biological product we have previously determined to ensure its effectiveness with clickers) [35]. The insects caught using traps and trapping belts trapped were counted, and the degree of infestation of adults and larvae by entomopathogens was determined in lab tests.

Field trials with the codling moth was carried out in the apple orchards of the Kuban Uchkhoz (Trubilin Kuban State Agrarian University). Infected insects were caught on a 1-hectare area. The number of captured infected insects were compared to that of the control (pesticide-treated) plots located at a distance of at least 500 m from the test plots.

Entomophages were isolated from the codling moth caterpillars caught with hunting belts followed by individual hatching.

The collected biomaterial was identified using the fundamental keys of the Zoological Institute RAS (St. Petersburg) and Far East Branch RAS (Vladivostok) [37, 38]. MLR 35 OH climate chambers were used to keep caterpillars and pupae of the codling moth to ensure either the emergence of entomophages from infected insects, or the emergence of butterflies. Microscopy was performed using MBS-10 binocular microscope (LZOS, Russia, 8× magnification).

Experimental data were statistically processed according to Dospekhov [39] using the Statistica 12.6 program (StatSoft, Inc., USA). The tables and figures show the means (*M*) and standard errors of the mean (±SEM). The significance of differences between the options was determined using the Student's *t*-test at $P \geq 0.95$.

Results. Lab screening the FNCBZR collection of entomopathogenic nematodes from various biotopes of the Krasnodar Territory reveled that three species (*St. carpocapsae*, *St. feltiae*, and *St. kraussei*) had the highest activity towards *G. pomonella* caterpillars and two species (*St. carpocapsae* var. "*agriotes*" and *St. feltiae*) towards wireworms [35, 36]. These species were involved in further studies.

Entomopathogenic nematodes are quite widespread in some biocenoses, and their main habitat is soil. Thereof, before studying the effect of introduced pathogens on the aboriginal pathogens of insects, we examined soils from biotopes in the experimental sites for the presence of entomopathogenic nematodes. In the apple orchard of FNCBZR intensively exploited for several years, we found *St. carpocapsae* and *Steinernema* sp. of the family *Steinernematidae*, hence, for dissemination we used *St. feltiae* nor found in the ecosystem that was chosen. According to our observations and data obtained earlier [40], a low number or almost complete absence of these pathogens are characteristic of row crops in crop rotation. A similar situation was seen in 2013 in the garden of the Educational farm Kuban, which was not used for 2 years before the research. I.e., the number of *St. carpocapsae* and *Steinernema* sp., caught with a bait insect, was lower here than in the FNCBZR orchard.

The test autodissemination of EPN against click beetles showed that the Kuban click beetle poses the greatest danger to the seedlings of soybeans and maize. In pheromone traps the number of beetles caught on maize and soybeans was 405.0±3.5 and 231.3±5.7, respectively, in 2011, 275.3±8.3 and 109.4±7.6 in 2012, and 119.7±7.6 and 86.7±7.6 in 2013 (Fig. 1). The abundance of common click beetle *A. sputator* was lower, 101.3±2.4 and 65.7±6.2 on maize and soybean in 2011, 76.6±6.0 and 38.7±3.6 in 2012, 42.0±5.6 and 32.0±3.6 in 2013 (see Fig. 1). The number of caught males of the steppe wireworm *A. gurgistanus* was minimum, 7.6±2.5 and 5.0±1.7 on maize and soybeans, respectively, in 2011, 6.3±1.5 and 3.7±0.6 in 2012, and decreased to 2.6±1.5 and 1.3±0.5 in 2013 (see Fig. 1).

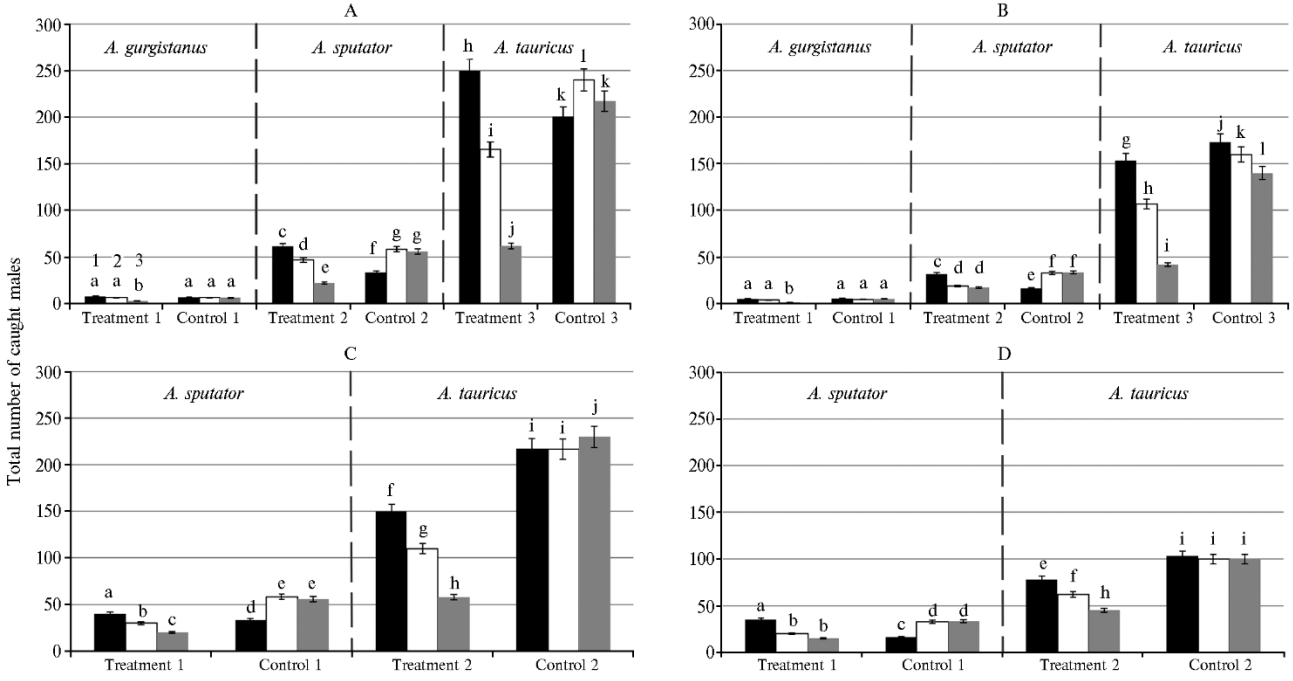

Fig. 1. Number of *Agriotes* click beetles in crops due to autodissemination: A and B — nematode *Steinernema carpocapsae* (maize and soybeans, respectively), C and D — nematode *St. feltiae* (maize and soybeans, respectively); I-III — application of nematodes and controls without application; 1, 2, 3 — 2011, 2012, and 2013 ($n = 10$, $M \pm$SEM, crop rotation, FNCBZR, Krasnodar Territory). Different letters mark statistically significant differences at $P \geq 0.95$. For the experimental design, see the *Materials and methods* section.

1. Efficiency of entomopathogenic nematodes *Steinernema carpocapsae* and *St. feltiae* towards *Agriotes* click beetles upon autodissemination in crops of maize and soybeans (n = 10, M±SEM, crop rotation, FNCBZR, Krasnodar Territory)

Click beetles	Nematodes	Number of caught males								
		2011			2012			2013		
		total	infested		total	infested		total	infested	
			total	%		total	%		total	%
		Maize								
A. sputator	*St. carpocapsae*	73.0±7.0	65.0±5.6	89.0	54.0±5.6	54.0±5.6	90.0	55.0±4.6[a]	47.0±6.0	85,4
Conrol		63,0±4,6	0	0	75.0±6.0	0	0	87.0±5.0	0	0
A. sputator	*St. feltiae*	40.0±5.0	32.0±3.5	80.0	26.0±1.8	26.0±1.8	86.7	23.0±2.6[c]	20.0±3.3	86,9
Conrol		40,0±4,0	0	0	50.0±5.5	0	0	60.0±6.2	0	0
A. tauricus	*St. carpocapsae*	150.0±10.3	147.0±7.8	98.0	88.0±2.7	88.0±2.7	97.7	60.0±7.2[e]	57.0±5.2	95,0
Conrol		150,0±8,0	0	0	160.0±9.3	0	0	160.0±9.6	0	0
A. tauricus	*St. feltiae*	68.0±4.6	61.0±6.2	89.7	33.0±3.6	33.0±3.6	94.2	25.0±4.3[g]	23.0±4.3	92,0
Conrol		70,0±4,3	0	0	79.0±6.2	0	0	85.0±6.5	0	0
A. gurgistanus	*St. carpocapsae*	7.0±1.8	6.0±3.6	85.7	4.0±1.0	4.0±1.0	80.0	4.0±2.5[i]	3.0±1.2	75,0
Conrol		5,0±2,5	0	0	6.3±1.5	0	0	7.0±1.0	0	0
		Soybeans								
A. sputator	*St. carpocapsae*	30.0±6.1	27.0±4.3	90.0	23.0±3.6	23.0±3.6	92.0	15.0±2.6	15.0±2.6	83,3
Conrol		31,0±4,8	0	0	38.0±3.6	0	0	43.0±6.9	0	0
A. sputator	*St. feltiae*	17.0±3.6	15.0±2.8	88.2	8.0±1.6	8.0±1.6	80.0	7.0±1.6	7.0±1.6	87,5
Conrol		15,0±4,2	0	0	21.0±2.6	0	0	25.0±3.6	0	0
A. tauricus	*St. carpocapsae*	130.0±6.2	122.0±6.9	93.8	63.0±7.9	63.0±7.9	90	47.0±5.3	47.0±5.3	79,6
Conrol		130,0±7,9	0	0	146.0±8.5	0	0	150.0±6.6	0	0
A. tauricus	*St. feltiae*	50.0±4.9	45.0±5.3	90.0	26.0±4.0	26.0±4.0	92.8	21.0±2.1	21.0±2.1	95,4
Conrol		48.0±5.2	0	0	53.0±6.3	0	0	60.0±6.5	0	0
A. gurgistanus	*St. carpocapsae*	5.0±1.5	3.0±0.8	60.0	2.0±0.4	2.0±0.4	66.7	2.0±0.2	2.0±0.2	100
Conrol		4.0±1.3	0	0	6.0±1.8	0	0	5.0±1.3	0	0

Note. Controls — without application of nematode autodissemination method. Different letters indicate statistically significant differences in the number of caught insects between the tests and controls in different years and on different crops at $P \geq 0.95$.

Thus, when using the autodissemination method for 3 years, the number of insects caught in pheromone traps decreased while in the control, it either did not change, as for the steppe wireworm (in 2011-2013, 6.0±1.0-6.6±1.5 and 4.3±1.1-5.3±0.6 individuals caught on corn and soybeans), or increased, as for the Kuban click beetle. The Kuban click beetle increased in abundance from 430.0±8.3 in 2011 up to 457.3±6.4 in 2013 on maize, the number of common click beetle increased from 66.6±3.7 in 2011 to 111.4±3.9 specimens in 2013 om maize. On soybeans, the male common click beetle also increased in number from 32.3±1.2 in 2011 up to 66.6±5.7 in 2013 (see Fig. 1). There was also a slight decrease in the number of captured males of the Kuban click beetle in the control on soybeans (from 276.6±5.7 in 2011 to 240.0±9.6 in 2013), however, the differences with the test treatments remained statistically significant at $P \geq 0.95$ (see Fig. 1).

The imagoes died in 4-5 days, which created conditions for the spread of infection. The number of individuals infected with *St. carpocapsae* was up to 83.3-92.0% for *A. sputator*, 79.6-98.0% for *A. tauricus*, and 60.0-100% for *A. gurgistanus*, Infestation by *St. feltiae* occurred in 80.0-88.2% of *A. sputator* and 9.7-94.2% of *A. tauricus* (Table 1). The number of released invasive nematode larvae per insect was 8.8×10^4 for *A. sputator*, 9.1×10^4 for *A. gurgistanus*, and 1.25×10^5 for *A. tauricus*, which suggests the introduction of more than 10 million entomopathogens into the environment and EPN activation in natural populations.

Additional introduction of nematodes into the soil, according to our findings and as previously noted by Danilov et al. [41], can cause a change in insect ethology. In wireworms, larvae infected with nematodes crawl out to the soil surface, becoming, as a result, more accessible to entomophages (carnivorous ground beetles) and vertebrates.

Note, in 2011-2013, there was a decrease both in the number of male click beetles caught in pheromone traps and damage to maize and soybean plants by pests by 13.2% compared to the use of chemical insecticide Cruiser®, KS (Syngenta, Switzerland) for seed treatment. Larvae of click beetles were also not found in the soil excavations. These were the result of the dissemination of entomopathogenic nematodes in 2011-2013.

2. Efficiency of entomopathogenic nematodes *Steinernema carpocapsae* and *St. feltiae* upon autodissemination in apple orchard (n = 10, M±SEM, Krasnodar Territory, 2013-2015)

Treatment	Number of caught insects							
	total				infested by nematodes, %			
	phytophages		entomophages		phytophages		entomophages	
	Cydia pomonella	*Grapholitha molesta*	*Chrysoperla carnea*	*Hymenoptera*	*Cydia pomonella*	*Grapholitha molesta*	*Chrysoperla carnea*	*Hymenoptera*
Kuban orchard								
Test	20.0±1.7[a]	0	0	20.0±0.6[c]	30.3	0	0	0
Control	40.0±2.2[b]	0	0	10.0±0.8[d]	0	0	0	0
FNCBZR orchard								
Test	37.0±3.5[a]	99.0±3.5[e]	9.0±1.1[g]	62.0±1.9[i]	35.3	34.0	0	0
Control	45.0±3.3[b]	120.0±4.1[f]	2.0±0.6[h]	10.0±1.7[j]	0	0	0	0

Note. Controls — without application of nematode autodissemination method. Different letters indicate statistically significant differences in the number of caught insects between the tests and controls at $P \geq 0.95$.

EPN were also autodisseminated in apple orchards for 3 years in two plots with different levels of pre-application of chemicals. Our studies have shown the possibility of using nematodes for autodissemination against the codling moth, since codling moth butterflies *Cydia pomonella* infected with pathogens were found in the traps during monitoring of pest abundance in both orchard agrocenoses, and the eastern codling moth *Grapholitha molesta* (Busck, 1916) also in

the FNCBZR orchard (Table 2). The identified percentage of *Lepidoptera* infested by entomopathogens was approximately the same, 30.3-35.3%. The number of helminths released from one insect was 1×10^4 for *C. pomonella* and 1×10^3 for *G. molesta*.

In the orchards, as in the crop rotation, we revealed a decrease in the number of insects caught in traps. There was a decrease in fruit damage (by about 10%) compared to standard protection systems, given that even in ecological gardens at least 4-5 treatments with various chemicals are carried out [6].

Among the captured entomophages, the *Chrysoperla carnea* St. and *Hymenoptera* (Linnaeus, 1758), namely, *Ascogaster quadridentatus* Wesmael, 1835, *A. rufidens* Wesmael, 1835, *Microdus rufipas* Nees, 1814 of the family *Braconidae* Latreille, 1829, and *Liotryphon crassisetus* (Thomson, 1877), *L. caudatus* (Ratzeburg, 1848), *L. punctulatus* (Ratzeburg, 1848) of the family *Ichneumonidae* Latreille, 1802, we did not identify insects infected with nematodes.

In other words, certain groups of entomophages turned out to be tolerant to the effects of entomonematodes. Back in 2008, Danilov et al. [41] hypothesized that the constant use of EPN in an apple orchard for a number of years contributes to an increase in both the quantitative and qualitative diversity of the species composition of entomophages. Our study has confirmed this hypothesis. The number of representatives of *Hymenoptera* identified in the second half of August was significantly higher. In the FNCBZR orchard where the method was tested we recorded an increase in the species diversity of *Hymenoptera* (from four species to six species) and the infection rate of *C. pomonella* caterpillars from 6% to 15% (Fig. 2).

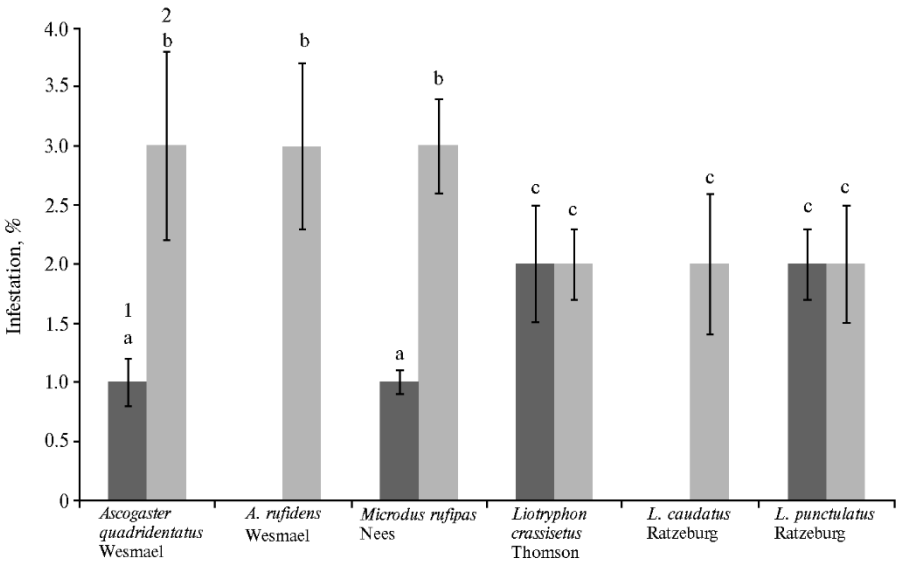

Fig. 2. Infestation of *Cydia pomonella* by entomophages of the families *Braconidae* and *Ichneumonidae* (*Hymenoptera*) in 2013 (1) **and 2015** (1) (100 insects in total, four repetitions; *M*±SEM, the FNCBZR orchard, Krasnodar Territory). Different letters indicate statistically significant differences in the number of entomophages between the years and $P \geq 0.95$.

A number of works [16, 42, 43] report that entomopathogenic fungi and viruses are agents mainly and quite successfully used for autodissemination in traps of various types (feromon, light, etc.). The entomopathogenic nematodes are traditionally applied by spraying, irrigation, treatment of the soil prior to crop sowing and near-tree rings in orchards, etc. [21, 44, 45]. The method of biological control of pests by means of EPN autodissemination that we propose in this paper is

another technique to use these entomopathogens.

Our research has demonstrated beneficial effect of the autodissemination of EPNs on increasing the invasive activity of natural populations of entomohelminths in the apple orchard. Soil biotests with *G. melonella* showed an increase in the number of invasive EPN larvae of the species *St. carpocapsae* per caterpillar two months after autodissemination compared to that prior to our experiment (Table 3). Danilov et al. [41] reported about an increased activity of local populations of pathogens upon the introduction of new species into the apple orchard agrocenosis, but these authors applied biologicals based on suspensions of entomopathogenic nematodes to near-tree rings. There are data, for example, reported by Somasekhar et al. [46], on the positive effect of introduced steinermatids on aboriginal nematode species of soils in agrocenoses.

In experiments, we also detectedhe nematode *St. carpocapsae* in the soil under the grain-row crop rotation, and pathogens persisted not only during the entire period of the study. As a result, stable foci of infection emerged, acting for several years (see Table 3).

Comparison of the number of *St. carpocapsae* caught in test to that under traditional protection from pests showed positive dynamics, while this did not occur in the control (see Table 3).

3. Number of *Steinernema carpocapsae* larvae caught in soil (bio-test with *Galleria melonella*) **upon autodissemination of nematodes of the *Steinernematidae* family** (*M*±SEM, Krasnodar Territory, 2011-2015)

Conditions	Depth, cm	
	5	10
Crop rotation of FNCBZR (2011-2013)		
Prior to the experiment	0	0
After the experiment:		
in 2 months	50.0±4.2	20.0±2.3
in 1 year	40.0±2.1	20.0±1.5
Control (without autodissemination)	0	0
Kuban orchard (2013-2014)		
Prior to the experiment	20.0±2.6	0
After the experiment:		
in 2 months	50.0±3.1	30.0±1.8
Control (without autodissemination)	17.0±2.2	0
FNCBZR orchard (2014-2015)		
Prior to the experiment	90.0±3.3	50.0±2.9
After the experiment:		
in 2 months	150.0±4.0	100.0±3.9
in 1 year	140.0±4.6	110.0±4.1
Control (without autodissemination)	80.0±3.3	40.0±1.9

Thus, we have found out that autodissemination of entomopathogenic nematodes of the *Steinernematidae* family are suitable to protect maize, soybeans and apple orchards from a various pests. Entomopathogenic nematodes disseminated by autodissemination infected 60.0-100% of male click beetles of the *Elateridae* family and 30.3-5.5% of apple and eastern moth butterflies, that is, the proposed method stands along with traditional methods of introducing pathogens in agroecosystems, especially in an organic garden where all chemicals are prohibited. Importantly, both in an apple orchard and crop rotations, the autodissemination methods affect certain groups of insects without causing harm to beneficial organisms. In addition, in all areas where autodissemination tests were carried out, the introduction of a species of pathogen into the agrocenosis favorably influenced on the invasive activity of local populations of entomopathogenic nematodes.

REFERENCES

1. Ismailov V.Ya., Agas'eva I.S. *Agro KHKHI*, 2003, 1-6: 72-74 (in Russ.).
2. Kirsch Ph. Pheromones: their potential role in control of agricultural insect pests**.** *American Journal of Alternative Agriculture*, 1988, 3(2-3): 83-97 (doi: 10.1017/S0889189300002241).
3. Płuciennik Z. The control of codling moth (*Cydia pomonella* L.) population using mating disruption method. *Journal of Horticultural Research*, 2013, 21(1): 65-70 (doi: 10.2478/johr-2013-0009).
4. Stelinski L.L., Gut L.J., Haas M., McGhee P., Epstein D. Evaluation of aerosol devices for simultaneous disruption of sex pheromone communication in *Cydia pomonella* and *Grapholita molesta* (*Lepidoptera*: *Tortricidae*). *Journal of Pest Science*, 2007, 80(4): 225-233 (doi: 10.1007/s10340-007-0176-7).
5. Vernon R.S., Toth M. Evaluation of pheromones: a new trap for monitoring *Agriotes lineatus* and *Agriotes obscurus* in the Fraser Valley of British Columbia. *Journal of Chemical Ecology*, 2007, 33(2): 345-351 (doi: 10.1007/s10886-006-9217-6).
6. Nadykta V.D., Niyazov O.D., Ivanova T.S., Pastarnak I.N., Pachkin A.A. *Politematicheskii elektronnyi zhurnal KubGAU*, 2012, 1(36): 106-109 (in Russ.).
7. Charmillot P.-J., Degen Th., Pasquier D., Briand F. New pheromone-based methods for grape moth control. *Journal of Pest Science*, 2005, 73(5): 283-288 (doi: 10.1007/s10886-016-0722-y).
8. Tokumaru S., Abe Y. Interspecific hybridization between *Liriomyza sativae* Blanchard and *L. trifolii* (Burgess) (*Diptera*: *Agromyzidae*). *Applied Entomology and Zoology*, 2005, 40(4): 551-555 (doi: 10.1303/aez.2005.551).
9. Howse P., Armsworth C., Baxter I. Autodissemination of semiochemicals and pesticides: a new concept compatible with the sterile insect technique. In: *Area-wide control of insect pests*. M.J.B. Vreysen, A.S. Robinson, J. Hendrichs (eds.). Springer, Dordrecht, 2007: 275-281 (doi: 10.1007/978-1-4020-6059-5_26).
10. Shamshev I.V., Grichanov I.Ya. *Zashchita i karantin rastenii*, 2008, 7: 22-23 (in Russ.).
11. Tsvetkova V.P., Poddubnaya E.N., Ismailov V.Ya., Zhuravlev S.V. *Vestnik Novosibirskogo GAU*, 2008, 7: 12-22 (in Russ.).
12. Sukovata L., Czokajlo D., Kolk A., lusarski Sł., Jabłoński T. An attempt to control *Cameraria ohridella* using an attract-and-kill technique. *Journal of Pest Science*, 2010, 11: 111-115 (doi: 10.1007/s10340-010-0342-1).
13. Sumedrea M., Marin F.C., Calinescu M., Sumedrea D., Iorgu A. Researches regarding the use of mating disruption pheromones in control of apple codling moth — *Cydia pomonella* L. *Agriculture and Agricultural Science Procedia*, 2015, 6: 171-178 (doi: 10.1016/j.aaspro.2015.08.055).
14. Sugonyaev E.S., Doroshenko T.N., Yakovuk V.A., Balakhnina I.V., Shevchenko O.S., Vasil'eva L.A. *Nauka Kubani*, 2010, 2: 42-47 (in Russ.).
15. Dzhadd G.Dzh.R. *Materialy Mezhdunarodnoi nauchno-prakticheskoi konferentsii «Biologicheskaya zashchita rastenii — osnova stabilizatsii agroekosistem»* [Proc. Int. Conf. «Biological plant protection as the basis for sustainable agroecosystems»]. Krasnodar, 2006, vyp. 4: 92-93 (in Russ.).
16. Ismailov V.Ya., Pachkin A.A., Agas'eva I.S. *Materialy Mezhdunarodnoi nauchno-prakticheskoi konferentsii «Biologicheskaya zashchita rastenii — osnova stabilizatsii agroekosistem»* [Proc. Int. Conf. «Biological plant protection as the basis for sustainable agroecosystems»]. Krasnodar, 2012, vyp. 7: 221-224 (in Russ.).
17. Danilov L.G. *Metodicheskie podkhody k izucheniyu osobennostei biologii entomopatogennykh nematod (Rhabditida: Steinernematidae, Haterorhabditidae) i tekhnologicheskie resheniya ikh effektivnogo ispol'zovaniya v kachestve sredstv biologicheskoi zashchity rastenii* [Methodological approaches to the study of the biology features of entomopathogenic nematodes (*Rhabditida*: *Steinernematidae*, *Haterorhabditidae*) and technologies for their effective use as biological plant protection agents]. St. Petersburg, 2003 (in Russ.).
18. Shternshis M.V. *Entomopatogeny — osnova biopreparatov dlya kontrolya chislennosti fitofagov* [Entomopathogens as the basis of biologicals to control the number of phytophages]. Novosibirsk, 2010 (in Russ.).
19. Mohan Sh. Entomopathogenic nematodes and their bacterial symbionts as lethal bioagents of Lepidopteran pests. *Biocontrol of Lepidopteran Pests*, 2015, 43(1): 273-288 (doi: 10.1007/978-3-319-14499-3_13).
20. Divya K., Sankar M. Entomopathogenic nematodes in pest management. *Indian Journal of Science and Technology*, 2009, 2(7): 53-60 (doi: 10.17485/ijst/2009/v2i7/29499).
21. Spiridonov S.E. V sbornike: *Prikladnaya nematologiya* /Pod redaktsiei S.V. Zinov'eva, V.N. Chizhova [In: Applied nematology. S.V. Zinov'ev, V.N. Chizhov (eds.)]. Moscow, 2006: 291-311 (in Russ.).
22. Shapiro-Ilan D.I., Morales-Ramos J.A., Rojas M.G. In vivo production of entomopathogenic nematodes. *Methods in Molecular Biology*, 2016, 1477: 137-158 (doi: 10.1007/978-1-4939-6367-6_11).
23. Koneru S.L., Salinas H., Flores G.E., Hong R.L. The bacterial community of entomophylic nematodes and host beetles. *Molecular Ecology*, 2016, 25(10): 2312-2324 (doi: 10.1111/mec.13614).

24. Danilov L.G. V sbornike: *Gel'minty nasekomykh* [In: Helminths of insects]. Moscow, 1980: 42-46 (in Russ.).
25. Danilov L.G., Airapetyan V.G. *Zashchita i karantin rastenii*, 2012, 8: 39-43 (in Russ.).
26. Liu J., Poinar G.O. Jr., Berri R.E. Control of insect pests with entomopathogenic nematodes: the impact of molecular biology and phylogenetic reconstruction. *Annual Review of Entomology*, 2000, 45(1): 287-306 (doi: 10.1146/annurev.ento.45.1.287).
27. Spiridonov S.E. V knige: *Patogeny nasekomykh: strukturnye i funktsional'nye aspekty* /Pod redaktsiei V.V. Glupova [In: Pathogens of insects: structural and functional aspects. V.V. Glupov (ed.)]. Moscow, 2001 (in Russ.).
28. El'-Dib A.M.A. *Vestnik Rossiiskogo universiteta druzhby narodov. Seriya: Agronomiya i zhivotnovodstvo*, 2010, 3: 24-28 (in Russ.).
29. Navaneethan Th., Strauch O., Besse S., Bonhomme A., Ehlers R.-U. Influence of humidity and a surfactant-polymer-formulation on the control potential of the entomopathogenic nematode *Steinernema feltiae* against diapausing codling moth larvae (*Cydia pomonella* L.) (*Lepidoptera*: *Tortricidae*). *BioSontrol*, 2010, 55(6): 777-788 (doi: 10.1007/s10526-010-9299-5).
30. Cruz-Martínez H., Ruiz-Vega J., Matada Mas-Ortíz P.T., Cortés-Martínez C.I., Osas-Diaz J.R. Formulation of entomopathogenic nematodes for crop pest control — a review. *Plant Protection Science*, 2017, 53(1): 15-24 (doi: 10.17221/35/2016-PPS).
31. Shternshis M.V., Andreeva I.V., Tsvetkova V.P. *Vestnik Novosibirskogo GAU*, 2011, 1(17): 7-12 (in Russ.).
32. Lavelle P. Diversity of soil fauna and ecosystem function. *Biology International*, 1996, 33: 3-16.
33. Yeates G.W. Nematodes as soil indicators: functional and biodiversity aspects. *Biology and Fertility of Soils*, 2003, 37(4): 199-210 (doi: 10.1007/s00374-003-0586-5).
34. Grubisic D., Gotlin T., Juran I. Biological control of codling moth, *Cydia pomonella* Linnaeus 1785 (*Lepidoptera*: *Tortricidae*) using entomopathogenic nematode *Steinernema carpocapsae* Weiser 1955 (*Rhabditida*: *Steinernematidae*). *Entomologia Sroatica*, 2010, 14 (3-4): 63-74.
35. Ismailov V.Ya., Pushnya M.V. *Materialy Mezhdunarodnoi nauchno-prakticheskoi konferentsii «Biologicheskaya zashchita rastenii — osnova stabilizatsii agroekosistem»* [Proc. Int. Conf. «Biological plant protection as the basis for sustainable agroecosystems»]. Krasnodar, 2012, vyp. 7: 228-230 (in Russ.).
36. Agas'eva I.S., Pushnya M.V. *Materialy Vserossiiskogo nauchno-prakticheskogo soveshchaniya «Ekologicheski bezopasnye i bespestitsidnye tekhnologii polucheniya rastenievodcheskoi produktsii»* [Proc. All-Russian Meeting «Environmentally friendly and pesticide-free technologies for crop production»]. Krasnodar, 1994: 220-223 (in Russ.).
37. Atanasov A.Z. V knige: *Opredelitel' nasekomykh evropeiskoi chasti SSSR* /Pod redaktsiei A.Z. Atanasova, V.P. Ionaitisa, D.R. Kasparyana, V.S. Kuslitskogo, A.P. Rasnitsyna, U.V. Siitana, V.P. Tolkanitsa [In: Keys to insects of the European part of the USSR. Vol. III. Part 3. A.Z. Atanasov, V.P. Ionaitis, D.R. Kasparyan, V.S. Kuslitskii, A.P. Rasnitsyn, U.V. Siitan, V.P. Tolkanits (eds.)]. Leningrad, 1981, Tom III, Chast' 3 (in Russ.).
38. Belokobyl'skii S.A. V knige: *Opredelitel' nasekomykh Dal'nego Vostoka Rossii* /Pod redaktsiei S.A. Belokobyl'skogo, V.I. Tobiasa [In: Keys to insects of the Russian Far East. Vol. IV, Part 4. S.A. Belokobyl'skii, V.I. Tobias (eds.)]. Vladivostok, 2000, Tom IV, Chast' 4 (in Russ.).
39. Dospekhov B.A. *Metodika polevogo opyta* [Methods of field trials]. Moscow, 1985 (in Russ.).
40. Ivakhnenko O.A., Ivanova T.S. *Materialy Mezhdunarodnoi nauchno-prakticheskoi konferentsii «Biologicheskaya zashchita rastenii — osnova stabilizatsii agroekosistem»* [Proc. Int. Conf. «Biological plant protection as the basis for sustainable agroecosystems»]. Krasnodar, 2006, vyp. 4: 234-235 (in Russ.).
41. Danilov L.G., Makhotkin A.G., Vasil'ev S.V., Turitsin V.S. *Parazitologiya*, 2008, 42(1): 129-137 (in Russ.).
42. El-Sufty R., Al Bgham S., Al-Awash S., Shahdad A., Al Bathra A. A trap for auto-dissemination of the entomopathogenic fungus *Beauveria bassiana* by red palm weevil adults in Date Palm Plantations. *Egyptian Journal of Pest Control*, 2011, 21(2): 271-276.
43. Benvenuti C., Barzanti G.P., Marianelli L., Sabbatini Peverieri G., Paoli F., Bosio G., Venanzio D., Giacometto E., Roversi P.F. A new device for auto-disseminating entomopathogenic fungi against *Popillia japonica*: a study case. *Bulletin of Insectology*, 2019, 72(2): 219-225.
44. Shapiro-Ilan D.I., Han R., Dolinksi C. Entomopathogenic nematode production and application technology. *Journal of Nematology,* 2012, 44(2): 206-217.
45. Cortés-Martínez C.I., Chavarría-Hernández N. Production of entomopathogenic nematodes in submerged monoxenic culture: a review. *Biotechnology and Bioengineering*, 2020, 117(12): 3968-3985 (doi: 10.1002/bit.27515).
46. Somasekhar N., Grewal P.S., De Nardo E.A.B., Stinner B.R. Non-target effects of entomopathogenic nematodes on the soil nematode community. *Journal of Applied Ecology*, 2002, 39(5): 735-744 (doi: 10.1046/j.1365-2664.2002.00749.x).