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The Application of Entomophagous and Acariphagous Species in Biological Protection Systems of an Apple Orchard (*Malus domestica* Borkh)

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Abstract: The systematic and long-term use of pesticides in fruit plantations leads to the formation of resistant pest populations. The purpose of this work was to evaluate the effectiveness of the use of entomophages and acariphages for the protection of apple orchards. Against the dominant pest *Cydia pomonella* (Linnaeus), *Habrobracon hebetor* (Say) was used, which was caught in the Krasnodar Territory using cassettes with caterpillars attractive to *H. hebetor*. To determine the most genetically high-quality population, an RAPD analysis was carried out from three Russian (Krasnodar, Stavropol, and Belgorod) and one Kazakh (Shymkent) populations of *H. hebetor*, which revealed a high level of DNA polymorphism and genetic diversity in the studied geographical populations of the cities of Krasnodar and Stavropol. The efficiency of the captured Krasnodar population of *H. hebetor* against *C. pomonella* was about 75%. To regulate the number of aphids *Aphis pomi* De Geer and *Tetraneura caerulescens* (Pass.), breeding reserves of the aphidophages *Harmonia axyridis* Pallas, *Leis dimidiata* Fabr., *Cycloneda sangvinea* L., and *Aphidius colemani* Vier. were established. The biological efficiency of the developed technique was 82.8–88.6%. The release of the acariphages *Amblyseius andersoni* (Chant) and *Metaseiulus occidentalis* (Nesb.) on the apple tree showed effectiveness from 80 to 90% against *Tetranychus urticae* Koch and *Panonychus ulmi* (Koch). To study the possibility of simultaneous use of entomophages and insecticides, experiments were carried out to study the sensitivity of *H. hebetor* and *H. axiridis* to insecticides. When *H. hebetor* cocoons were treated with Insegar® and Atabron®, the ectoparasitoid emergence values were 98.4% and 100%, respectively. The survival of adult *H. axiridis* treated with Madex twin®, Atabron®, and Koragen® on the fifth day was 97.3%, 89.6%, and 81.9%, respectively. Based on the data obtained, it can be argued that it is possible to create favorable conditions for entomophages, which effectively regulate pest numbers in apple orchards.

Keywords: biological protection; aphidophagous species; codling moth; *Habrobracon hebetor* (Say)

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1. Introduction

The apple tree (*Malus domestica*), a member of the Rosaceae family, is one of the oldest cultivated fruit crops, having been grown for over 5000 years. According to FAO estimates, today it is the basis of fruit growing in 86 countries; the total cultivated area is 4.6 million hectares, and the annual yield is about 86.5 million tons of apples [1]. As a result of such a long cultivation, there are more than 400 pest species of the apple tree. Many of these phytophagous species do not cause significant crop damage. The main threat is posed by dominant pest species, which have become so due to their high abundance and harmfulness [2]. Due to the overwhelming globalization process, many dominant apple pests have become super-dominant, posing problems for apple growers worldwide [3].

Super-dominant apple pests include the following species: *Cydia pomonella* (Linnaeus, 1758) (Lepidóptera: Tortricidae); aphids such as *Aphis pomi* De Geer, 1773, and *Tetraneura caerulescens* Passerini, 1856 (Homoptera: Aphidoidea); and herbivorous mites, in particular

Tetranychus urticae Koch, 1836, and *Panonychus ulmi* (Koch, 1836) (Trombidiformes: Tetranychidae). Each of these pests causes considerable yield damage. Therefore, it is essential to introduce effective crop protection methods to combat these pests. The use of insecticides is one of them. However, systematic and long-term use of pesticides in fruit plantations leads to the development of resistant pest populations [4–6]; disruption of natural biocenotic regulation as a result of the death of entomophagous and acariphagous species also occurs. That is, the effectiveness of pesticides is constantly decreasing. Note that it still does not reach 70–80% even with an increase in consumption rates and the frequency of treatments [7]. In addition, many consumers choose organic food due to perceived health benefits and reduced environmental impacts [8,9]. Therefore, organic farming and an integrated protection system are actively developing, allowing the combined use of biorational preparations and entomophagous species. An advantageous pest management scheme in organic crop production requires biological methods of plant protection. The selection of environment-friendly biorational preparations that restore the balance between harmful and beneficial species is necessary as well.

Studies of fruit production in 85 apple orchards in three European countries have shown that organic orchards had numbers of entomophagous and acariphagous species 38% higher than integrated pest management (IPM) orchards [10]. To date, cases of successful control of the number of harmful insects and mites with the help of entomophagous and acariphagous species are known. For example, in Poland, high levels of hymenoptera parasitism were noted (rose tortrix, *Archips rosana*, Linnaeus, 1758) [11]. The ectoparasitoid *H. hebetor* is one of the most widely used biological controllers in biological plant protection against harmful lepidoptera, including extremely harmful pests of corn, soybean, vegetable crops, and fruit crops such as apple [12]. In Washington apple orchards, entomophagous species capable of controlling the number of woolly apple aphids (*Eriosoma lanigerum* (Hausmann, 1802)) were identified. The most common predators were from the families Syrphidae, Chrysopidae, and Coccinellidae; among parasitoids, *Aphelinus mali* (Haldeman, 1851) was noted [13]. *Harmonia axyridis* Pallas, 1773 (Coleoptera: Coccinellidae), native to eastern Asia, is one of the most important predatory beetles used throughout the world in the biological control of insect pests on crops [13]. In Turkish apple orchards, the aphid feeders *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae) and *Coccinella semptempunctata* Linnaeus, 1858 (Coleoptera: Coccinellidae), effectively control the number of aphids [14]. In Washington apple orchards, complex control of mites is based on the conservation of natural enemies, especially phytoseiid mites [15]. The predatory mites *Phytoseiulus persimilis* Athias-Henriot, 1916, *Amblyseius andersoni* (Chant, 1957), and *Amblyseius californicus* (McGregor, 1954) control the abundance of herbivorous mites on apple trees in Crimea [16]. Several species of phytoseiidae mites (Acari: Phytoseiidae) from the genera *Amblyseius*, *Galendromus*, *Metaseiulus*, *Neoseiulus*, *Phytoseiulus*, and *Typhlodromus* are now grown for biological control of various crop pests [17].

This paper describes the experience of using entomophagous and acariphagous species in apple orchards with an organic type of protection. The local ecotype of the hymenopteran ectoparasitoid *H. hebetor* was used against *C. pomonella*. Several species of ladybugs (Coccinellidae) and the hymenopterous parasite *Aphidius colemani* Viereck, 1912 (Hymenoptera: Braconidae), were applied to control *A. pomi* and *T. caerulescens*. The acariphagous species *A. andersoni* and *M. occidentalis* (Mesostigmata: Phytoseiidae) were used to suppress outbreaks of the herbivorous mites *T. urticae* and *P. ulmi*. The compatibility of insecticides and certain entomophagous species is considered. The laboratory-reared population of insects (*H. hebetor*) is assessed using genetic analysis.

Artificially cultivated entomophagous species contribute to the reproduction of natural populations of parasitic and predatory arthropods. This is key to the formation of a stable cenosis of an apple orchard [18].

Quality control of insect mass cultures is crucial to production efficiency. There is an express method for assessing the quality of uterine cultures of entomophagous species artificially grown for mass release into agrocenoses. This method is based on the assessment

of genetic polymorphism and diversity of insect populations by DNA markers (RAPD and ISSR) and can be viewed as a quality criterion for commercial batches of bioagents. This, in turn, can significantly increase the effectiveness of the biological protection of agricultural plants. In a practical aspect, we propose to consider the manifestation of population heterogeneity as a criterion for the viability of a population and use it to predict the population dynamics and control the quality of the biomaterial.

We assume that the use of entomophagous and acariphagous species in apple orchards will effectively control the number of pests at a level not exceeding the economic threshold. We, therefore, aim to assess the effectiveness of the use of entomophagous and acariphagous species in biological protection systems of apple orchards.

2. Materials and Methods

The effectiveness of entomophagous species in protecting apple trees from major pests was evaluated in organic orchards at the Shcherbakov farm (Krasnodar Krai, 45°05′ N 38°28′ E) with an area of 5 ha and the Kolt Tekhnologii farm (Krasnodar Krai, 44°56′ N 37°52′ E) with an area of 3 ha.

The research was carried out using the material and technical base of the USI (a unique scientific installation) "Technological line for the mass breeding of entomophagous species" No. 671922 https://ckp-rf.ru/catalog/usu/671922/ (accessed on 5 March 2023), as well as objects of the biological resource collection, BRC "State Collection of Entomoacariphagous and Microorganisms" of the Federal Research Center of Biological Plant Protection (FRC BPP), BRC GKEM registry No. 585858 https://www.fncbzr.ru/brk-i-unu/unique-installation-1/ (accessed on 5 March 2023).

Entomophagous and acariphagous species were produced in the Laboratory of the State Collection of Entomoacariphagous Species and Primary Evaluation of Biological Plant Protection Products and the Laboratory of Chemical Communication and Mass Breeding of Insects at the FRC BPP (Krasnodar).

An initial population of *H. hebetor* was captured in the FRC BPP apple orchard (45°02′ N 38°59′ E). In each of 5 trapping cassettes (Figure 1), we placed last instar caterpillars of *Galleria mellonella* (Linnaeus, 1758) (20 specimens) and *Ephestia kuhniella* Zeller, 1879 (30 specimens). The cassettes were then hung on the trees with the most windfall. The exposure time was 7 days. After that, the cassettes were replaced with new ones. The cassettes were hung out three times. Further, in the laboratory, the *H. hebetor* parasitoids were removed and bred.

Figure 1. Cassettes with codling moth caterpillars for catching *Habrobracon hebetor* in an apple orchard.

The effectiveness of the captured *H. hebetor* against *C. pomonella* was evaluated in the laboratory. For infestation, caterpillars of *C. pomonella* at various instar phases were offered to *H. hebetor*. An average of 30 to 45 codling moth caterpillars were placed in a glass vessel with a capacity of 0.7 L, and then 20 ectoparasitoids were introduced. Experiments were performed in triplicate. The effectiveness was assessed by counting paralyzed and parasitized caterpillars, as well as by the number of cocoons formed and emerged adults of *H. hebetor*.

Mass rearing of *H. hebetor* and its hosts, *E. kuhniella* and *G. mellonella*, was carried out on artificial nutrient media [19] according to a previously developed method [20–22].

The greenbug (*Schizaphis graminum* (Rondani, 1852)) served as feed for mass rearing of *H. axyridis*, *Leis dimidiata* Fabricius, 1781, *Cycloneda sanguinea* Linnaeus, 1763, and *A. colemani*.

Predatory mites were bred on *Acarus siro* Linnaeus, 1758, in the laboratory using bran, according to previously described methods [23].

The objects of molecular genetic studies were insects from the Russian (Krasnodar, Stavropol, Belgorod) and Kazakh (Shymkent) populations of *H. hebetor*. The sample of insects for each study site (for each locality) in triplicate was $n = 60$ ($20 \times 3 = 60$). A total of 240 insects were analyzed at four research sites ($60 \times 4 = 240$). DNA isolation was performed with adult insects using agarose gel electrophoresis-based RAPD-PCR (1.8%). Four highly specific *H. hebetor* DNA primers were used in the PCR reaction: OPA05, OPA10, ORB04, and UBC519 (Evrogen, Moscow, Russia). Gene Ruler 100 bp DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA) was used as a DNA molecular weight marker. Genetic diversity, DNA polymorphism, and genetic similarity were assessed by Nei and Shannon using the Popgene version 1.31 software [24].

The compatibility study of the ectoparasitoid *H. hebetor* and ladybug *H. axyridis* with biological and chemical preparations recommended for protecting the apple tree from the codling moth and other lepidoptera pests was carried out in glass containers with a volume of 0.7 l. Experiments were performed in triplicate. We placed 50 *G. mellonella* caterpillars and 25 adults of *H. hebetor* in each container to study the sensitivity of *H. hebetor*. Pupation was completed after 7 days; then, the formed cocoons were counted and treated with insecticides. In the *H. hebetor* sensitivity tests, the following products were used (the active substance and formulation of the insecticide are given in brackets): Lepidocid ® (*Bacillus thuringiensis* var. kurtsaki, suspended concentrate), FermoVirin/YP® (codling moth granulosis virus, wettable powder), Insegar® (phenoxycarb, water-dispersible granules), Atabron® (chlorfluazuron, suspended concentrate), and Decis Expert® (deltamethrin, emulsifiable concentrate). To study the susceptibility of coccinellidae, 26 adults of *H. axiridis* were placed in each container and then treated with the following products: Spintor ® (spinosad, suspended concentrate), Madex twin® (codling moth granulosis virus, suspended concentrate), Admiral® (piriproxifen, emulsifiable concentrate), Koragen® (chloranthraniliprol, suspension concentrate), Atabron® (chlorofluazuron, suspension concentrate), and Akkar® (*Verticillum lecanii*, *Hirsutella thompsonii*, *Beauveria bassiana*, *Bacillus thuringiensis*, liquid). The dosages were calculated according to the instructions.

The release of *H. hebetor* was carried out at the rate of 1200 individuals/ha at the Shcherbakov farm on an area of 5 ha. Biological efficiency was determined by the percentage of infected caterpillars in trapping belts and cassettes, in which caterpillars *G. mellonella* (15 specimens) and *C. pomonella* (15 specimens) were placed as an insect test object. In total, 10 cassettes were hung in the orchard.

The release of aphidophagous species was carried out at the Kolt Tekhnologii farm in the spring, when aphids of the species *A. pomi* and *T. caerulescens* were first detected in the half-inch green—tight-cluster phenophase coccinellids (*H. axyridis, L. dimidiata, C. sangvinea*) were released at a predator/prey ratio of 1:3–1:8; and *A. colemani* were released at a parasite/host ratio 1:10–1:15. The accounting was performed by counting adults and nymphs per 10 cm shoot (one branch from 4 sides of the canopy of each model tree; the branches were labeled). The counts were implemented on eight model trees. The

release of aphidophagous species was carried out annually. Table 1 presents the scheme of the experiment.

Table 1. Scheme of experiment in the use of aphidophagous and acariphagous species.

Option	Release Rate, Individuals/ha
aphidophages	
Coccinellidae (*Harmonia axyridis* Pallas, *Leis dimidiata* Fabr., *Cycloneda sangvinea* L.)—larvae	2000–3000
Aphidius colemani Vier.—imago	10,000
Control	without release
acariphagous	
Metaseiulus occidentalis Nesb. + *Amblyseius andersoni* Athias-Henriot	10,000–12,000
Control	without release

The predatory mites *M. occidentalis* and *A. andersoni* were released to control the tetranychus species. The effectiveness of predatory mites was assessed by counting nymphs and adults, using a 7–10-fold magnifier, on 20–40 leaves (5–10 leaves from each of the 4 sides of the canopy, depending on the degree of colonization) from each of the eight accounting trees.

Statistical data were processed using Student's t-test for small independent samples with a confidence interval of 0.05, as well as Duncan multiple range test using the Statistica 13.0 software.

3. Results

3.1. Laboratory Investigation of the Parasitism of H. hebetor Natural Population on C. pomonella

We have established that the density of the phytophagous population, the generations' number, the timing and duration of crop flowering, and the presence of other types of parasitic and predatory entomophagous species to determine the fertility, parasitic activity, and number of generations of *H. hebetor*. Therefore, we used the local ecotype to test in practice its ability to protect apple trees from the codling moth (Table 2).

Table 2. Parasitic activity of the ectoparasitoid *H. hebetor* against the codling moth *C. pomonella*.

Codling Moth, Caterpillars	Number of Caterpillars, Ind.	Including:		The Number of Cocoons Formed, Ind.	Number of Emerged Parasitoids, %
		Paralyzed, %	Parasitized, %		
old age	42 ^{e} *	100	75.6	33 ^{a}	78.6
middle age	34 ^{a}	100	68.7	20 ^{c}	54.8
young age	37 ^{d}	0	0	0 ^{b}	0

* Note: each letter represents a category. There are no statistically significant differences between the options indicated by the same letter indices when compared within the columns according to the Duncan criterion at a probability level of 95%.

Table 2 shows that under laboratory conditions *H. hebetor* paralyzes 100% of middle- and older-aged caterpillars of the codling moth. The emergence values of parasitoids in those groups were 54.8 and 78.6%, respectively. These are new results for other populations in the FRC BPP collection. Thus, we assume that the new leaf-roller population of *H. hebetor*, propagated under laboratory conditions, meets all the requirements for its use as a bioagent for codling moth control.

3.2. Molecular Genetic Analysis for *H. hebetor* Population Quality Assessment

We performed a molecular genetic analysis of the heterogeneity of four geographic populations of *H. hebetor* to assess their protection strategies in experimental apple orchards. The Kazakhstan and Belgorod populations had a relatively low level of DNA polymorphism: 26.4% and 35.9%, respectively. The same populations were also characterized by relatively low genetic diversity calculated from the Nei's and Shannon indices (Table 3). In the Krasnodar population, the level of genetic diversity was H = 0.22 ± 0.19; I = 0.33 ± 0.28, and the DNA polymorphism level was 64.2%.

Table 3. DNA polymorphism and genetic diversity of *H. hebetor* populations.

Sample from a Population	P (%)	Na ± SD *	Ne ± SD *	H ± SD *	I ± SD *
Belgorod	35.9	1.36 ± 0.48	1.22 ± 0.35	0.13 ± 0.19	0.19 ± 0.27
Chimkent	26.4	1.26 ± 0.45	1.16 ± 0.31	0.09 ± 0.17	0.14 ± 0.25
Stavropol	45.3	1.45 ± 0.50	1.26 ± 0.34	0.15 ± 0.19	0.23 ± 0.28
Krasnodar	64.2	1.64 ± 0.48	1.37 ± 0.35	0.22 ± 0.19	0.33 ± 0.28

* $t_{emp} \leq t_{crit}$—differences are not statistically significant; P—% polymorphic loci in a population; Na—the number of alleles per locus; Ne—the effective number of alleles per locus; H—genetic diversity according to Nei; I—Shannon information index; ±SD—standard deviation.

Genetic similarity analysis showed that the Russian population from Belgorod and the one from Kazakhstan are the most genetically close (genetic identity GI = 0.979; genetic distance GD = 0.022) (Table 4). At the same time, the two Russian populations from Krasnodar and Stavropol also turned out to be genetically close to each other (GI = 0.849; GD = 0.164).

Table 4. Genetic distances (GD) (under diagonal) and genetic identity (GI) (above diagonal) between populations of Habrobracon.

Sample from a Population	Belgorod	Chimkent	Krasnodar	Stavropol
Belgorod	–	0.979	0.770	0.789
Chimkent	0.022	–	0.743	0.736
Krasnodar	0.261	0.297	–	0.849
Stavropol	0.237	0.307	0.164	–

Subsequently, using all the identified DNA fragments, we conducted a cluster analysis of the *H. hebetor* populations (Figure 2).

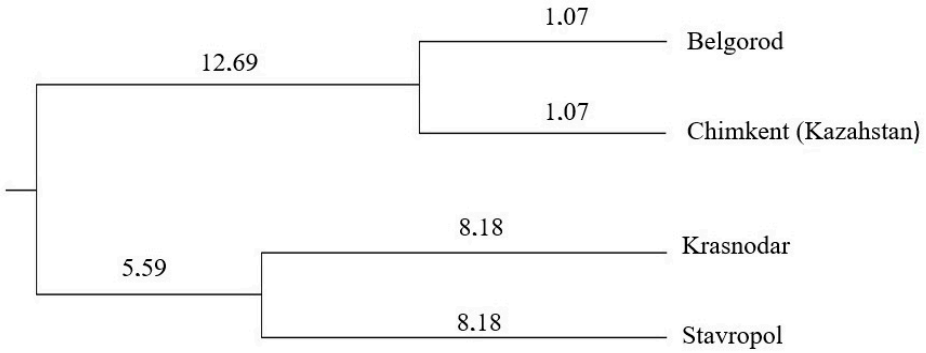

Figure 2. Dendrogram of Cluster Analysis of *H. hebetor* Geographic Populations.

The genetic diversity (the Nei's index) within the geographical samples from Belgorod and Chimkent (Hs = 0.109) is 92% of the total genetic diversity (Ht = 0.119). This indicates a significant level of gene flow between them. The calculated data confirm it: the level of genetic flow is Nm = 5.74. The relatively low value of the coefficient of genetic differentiation (Gst = 0.08) indicates that 8% of the total genetic variability falls on the share of variability between populations, which determines the differentiation between samples. For the Krasnodar and Stavropol samples, the internal genetic variability is Hs = 0.186 of the total genetic variability Ht = 0.248. The level of genetic flow between populations is Nm = 1.51; and the coefficient of genetic differentiation Gst = 0.25. This indicates that the share of intrapopulation variability accounts for 75%, while the share of interpopulation variability is 25%.

3.3. Sensitivity of Entomophagous Species to Biological and Chemical Insecticides

Traditional insecticides negatively affect the number and efficiency of natural and introduced populations of entomophagous species. Therefore, we studied in the laboratory the sensitivity of entomophagous species to insecticides used in apple orchards (Table 5).

Table 5. Susceptibility of *H. hebetor* to biological and chemical insecticides in a laboratory test, (M ± SD).

Preparation, Active Substance	Consumption, L/ha, kg/ha, g/ha	Cocoons before Treatment, Ind.	Imago Emergence				
			Upon the Time of Accounting, Ind.			Total, Ind.	From the Initial Number, %
			3rd Day	5th Day	7th Day		
Biological insecticides							
Lepidocid® (*Bacillus thuringiensis var. kurtsaki*)	2.0	69.2	10.2 ± 2.1	37.6 ± 1.6	7.4 ± 1.8	55.2	79.8 ^{c} *
FermoVirin/YP® (codling moth granulosis virus)	1.0	83.4	22.5 ± 3.7	39.6 ± 2.1	21.3 ± 3.4	83.4	100 ^{b}
Biorational insecticides							
Insegar® (phenoxycarb)	0.6	80.3	14.7 ± 1.5	51.9 ± 3.3	12.4 ± 1.1	79.0	98.4 ^{bc}
Atabron® (chlorfluazuron)	0.75	76.2	24.8 ± 3.2	44.3 ± 1.3	9 ±1.6	76.2	100 ^{b}
Chemical insecticides							
Decis Expert® (deltamethrin)	0.1	87.5	0	0	0	0	0 ^{a}
Control		93.0	20.9 ± 1.6	56.2 ± 4.2	13.9 ± 2.3	93.0	100 ^{b}

* Each letter represents a category. There are no statistically significant differences between the options indicated by the same letter indices within the column according to the Duncan criterion at a probability level of p = 95%.

The survival rate of *H. hebetor* after treatment with the biorational insecticides Insegar® and Atabron® was 98.4% and 100%, respectively. The Decis Expert® chemical resulted in the death of all treated insects. Lepidocid® preparation based on *Bacillus thuringiensis* made it possible to obtain 79.8% of viable adults. The biological product FermoVirin/YP® based on the codling moth granulosis virus proved to be non-toxic.

Table 6 shows data on the sensitivity of adult coccinellidae to biological and biorational preparations.

Table 6. Sensitivity of adult *H. axyridis* to biological and biorational insecticides.

Variant	Consumption, L/ha, kg/ha	Number of Beetles before Treatment, Ind.	After Treatment by Counting Days, Ind.			Surviving Individuals on Day 5, %
			1st Day	3rd Day	5th Day	
Spintor® (Spinosad)	0.5	26 ± 0.0	26 ± 0.0	25.7 ± 0.3	16.1 ± 1.9	61.9
Madex twin® (Codling moth granulosis virus)	0.1	26 ± 0.0	26 ± 0.0	25.7 ± 0.3	25.3 ± 0.7	97.3
Admiral® (Piriproxifen)	0.7	26 ± 0.0	26 ± 0.0	21.3 ± 1.7	13.0 ± 1	50.0
Koragen® (Chloranthraniliprol)	0.2	26 ± 0.0	26 ± 0.0	23 ± 1.0	21.3 ± 0.7	81.9
Atabron® (Chlorofluazuron)	0.7	26 ± 0.0	26 ± 0.0	25.7 ± 0.3	23.3 ± 0.7	89.6
Akkar®, (*Verticillum lecanii*, *Hirsutella thompsonii*, *Beauveria bassiana*, *Bacillus thuringiensis*)	5.0	26 ± 0.0	26 ± 0.0	24.7 ± 0.3	18.7 ± 0.3	71.9
Control	-	26 ± 0.0	26 ± 0.0	26 ± 0.5	26 ± 0.9	-

Let us note the high sensitivity of Harmonia to the juvenoid insecticide Admiral®, which amounted to 50.0%. The least sensitivity in coccinellidae was observed to Madex twin® (survival of adults was 97.3%).

3.4. Field Evaluations of the Efficacy of the Entomophagous Species in Pests Control

3.4.1. Habrobracon Hebetor to Control *C. pomonella*

We studied the effectiveness of the natural population of *H. hebetor* in the Shcherbakov farm. The count in the trapping belts showed the infestation of caterpillars of the codling moth prior to pupation (Table 7).

Table 7. The number of the infected caterpillars in trapping belts.

Variant	Number of Caterpillars, Ind.	Number of the Infected Caterpillars, Ind.	Number of Cocoons, Pcs.	Parasitized, %	The Number of the Emerged Adult Parasitoids, %
Parasite release	6.6	5.4	6.2	79.2	100
Control (without parasite release)	6.2	0.4	0.4	6.5	100

The hypothesis is rejected in the T-test at a critical significance level of 95%.

In trapping belts, most of the codling moth caterpillars were infected by *H. hebetor*. We were unable to find the wasp itself. However, according to the characteristic damage in caterpillars and the presence of empty *H. hebetor* cocoons next to them, it can be argued that an entomophagous species was active against the codling moth and successfully pupated. Therefore, a prolonged effect of pest control is possible. The average biological efficiency of the ectoparasitoid release was 72.3%.

3.4.2. Aphidophagous Predators to Control Aphids

Spring weather conditions in 2020–2021 were favorable for the development of the apple aphid *A. pomi* and the red gall aphid *T. caerulescens*. The number of aphidophagous predators—Coccinellidae, Chrysopidae, Aphidiinae, and others—was very low in early spring. As a result, aphids multiplied quickly and damaged young shoots. This led to twisting of the leaves and a change in their color (Figure 3a).

(**a**) (**b**)

Figure 3. Organic apple orchard at the Shcherbakov farm: (**a**)—apple tree leaves affected by aphids; (**b**)—release of ladybird larvae.

The release of aphidophagous species was carried out on trees where aphid reserves were found, which accounted for 10–15% of the total. In the apple orchard of the Kolt Tekhnologii farm, ladybug larvae were released into aphid pockets (Figure 3b). Their total number was 2000–3000 individuals/ha. In addition to Coccinellidae, mummies infected with the Aphidiinae *A. colemani* were placed in pockets with aphids throughout the entire orchard.

Table 8 shows that the average number of aphids on infested trees was 18.4–27.3 ind./shoot. The created reproducing reserves of aphidophagous species helped to restrain the harmfulness of aphids during the formation of leaves in early spring, when the density of the natural population of Coccinellidae was low. The method efficiency was 82.8–88.6%.

Table 8. Effectiveness of aphidophagous species against aphids (Shcherbakov farm, 2019–2020).

Year	Average Number of Aphids Per Shoot	
	Before Release of Aphidophagous Species, Ind.	3 Weeks after the Release, Ind.
2019	18.4 ± 1.5 *	2.1 ± 1.2
2020	27.3 ± 2.5	4.7 ± 1.9

* For every year the null hypothesis is rejected in the T-test at a critical significance level of 95%.

3.4.3. Application of Acariphagous Species to Suppress Tetraniquid Mite Populations during the 2019–2020 Growing Seasons; Two Dominant Species Were Identified on the Apple Tree: The Red Spider Mite *T. urticae* and the European Red Mite *P. ulmi*

For the bio-control of *T. urticae* and *P. ulmi* on apple trees, a mixture of the predatory mites *M. occidentalis* and *A. andersoni* was introduced into the natural foci of the prey as they were found. This made it possible to establish reproducing acariphagous reserves in advance.

A preliminary count of the number of mites before the spread of acariphagous species showed the presence of 10–15% of plants with a second colonization score (mite colonies occupy up to 25% of the leaf surface). The economic threshold of harmfulness of the spider mite on an apple tree is 4–5 mites per 1 leaf, with 10% of plants infested.

We conclude the positive effect of releasing predatory mites against nymphs and adults of the spider mite. Predatory activity on eggs was not observed. As a result, after 5–7 days, when the hatching of young mites began, predators were reintroduced (Table 9).

Table 9. Application results of the predatory *A. andersoni* and *M. occidentalis* against herbivorous mites on an apple tree in 2020.

Initial Number of Phytophagous Mites (Ind./Leaf)		The Number of Phytophagous Mites on a Certain Day (Ind./Leaf)					
		7 Day		14 Day		21 Day	
Mobile Stages	Eggs	Mobile Stages	Eggs	Mobile Stages	Eggs	Mobile Stages	Eggs
Number of spider mites, ind./leaf							
9.4 ^{d} *	5.7 ^{d}	7.9 ^{c}	4.3 ^{c}	3.7 ^{b}	2.1 ^{b}	1.6 ^{a}	0.4 ^{a}
Number of European red mites, ind./leaf							
6.2 ^{d}	3.8 ^{a}	5.0 ^{c}	3.3 ^{a}	3.3 ^{b}	1.8 ^{c}	1.3 ^{a}	0.5 ^{b}

* Each letter represents a category. There are no statistically significant differences between the variants indicated by the same letter indices when separately comparing motile stages and eggs according to the Duncan criterion at a probability level of 95%.

In 2021, the abundance of herbivorous mites was slightly lower than in 2019 where *A. andersoni* and *M. occidentalis* were released. Figure 4 shows that in early July, the abundance of herbivorous mites was 3.5 ind./leaf, both in the control and in the plots where the predators were released. Furthermore, the number of herbivorous mites in the control plot began to increase, while in the experimental plot it remained at the same level and then began to decrease. By August 10, the number of mites in the control plot was 7.5 ind./leaf, and 0.7 ind./leaf in the experimental plot. The acariphagous species efficiency was 90.7%.

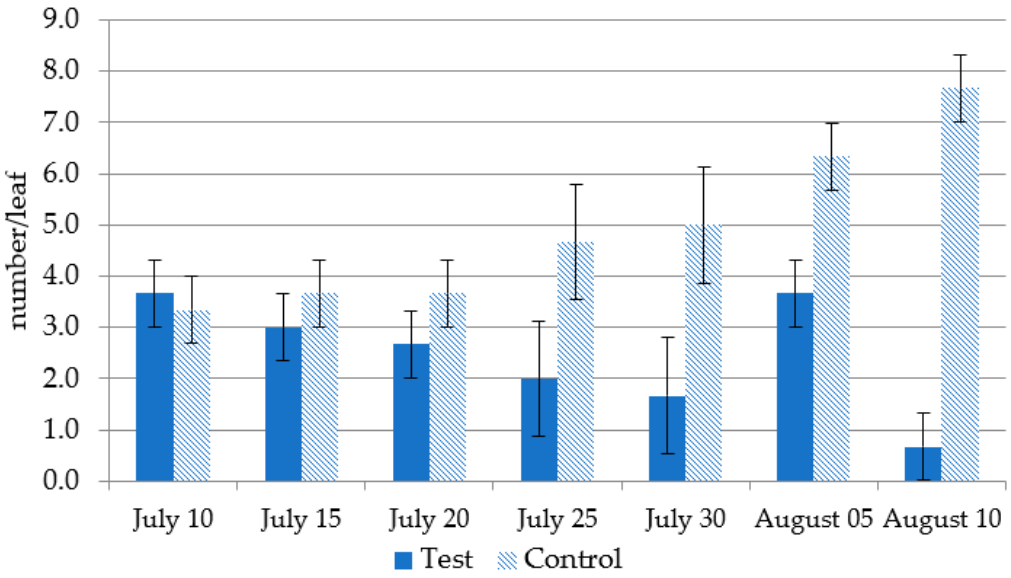

Figure 4. Change in the number of herbivorous mites due to the introduction of predatory phytoseiidae mites on an apple tree in 2021.

4. Discussion

Habrobracon hebetor is a promising potential biological control agent for the codling moth. For many years, it has been selected for breeding and used against more than 70 species of lepidoptera pests [25,26]. However, some papers indicate that its trophic relationships are much broader in both laboratory and field conditions. This complicates the practical application of *H. hebetor* in biological plant protection [27–29]. Capturing and studying the local ecotype gave encouraging results: the efficiency of the captured local population was higher compared to laboratory populations from the FRC BPP collection. In addition, the study of the trophic specialization of the parasite made it possible to

determine against which codling moth caterpillar instar it is most effective. Here we argue that ectoparasite release is ineffective for young codling moth caterpillars. The application of *H. hebetor* against middle-aged and older caterpillars controls pest numbers, as well as prolongs the protective effect through a second generation that flies out after infestation of middle-aged and older caterpillars. This effect may subsequently lead to natural biocenotic regulation in an apple orchard where *H. hebetor* is released.

Thanks to the wide development of technical entomology, it has become possible to artificially rear a large number of entomophages in biolaboratories in order to maintain, study, and select promising bioagents for biological plant protection programs. Insects grown under laboratory conditions are a rapidly reproducing object, and for the year-round maintenance of mother cultures, they require an assessment of the heterogeneity of the population structure, namely, indicators of genetic diversity and DNA polymorphism. In connection with the long-term rearing of entomophages, there is a decrease in these indicators caused by inbreeding as a result of laboratory conditions for their maintenance. To improve the quality of such laboratory populations, their renewal (hybridization) is required. In this work, we evaluate the heterogeneity of four samples of the ectoparasite *H. hebetor*. We have also analyzed populations of the aphidophage predator *H. axyridis*. The analyzed laboratory populations of the predator underwent regular hybridization with individuals from the natural population, as a result of which the heterogeneity of these populations was high. Therefore, in order to show the difference in heterogeneity, we present data on the assessment of DNA polymorphism and genetic diversity using the ectoparasite *H. hebetor* as an example. Thus, the analysis of the heterogeneity of populations of entomophages (both predatory and parasitic), based on the assessment of their DNA polymorphism and genetic diversity, is used as a quality criterion for commercial batches of bioagents, which can significantly increase the efficiency of mass breeding and the use of entomophages in pest control [30].

Genetic similarity analysis allows the identification of the mixing of races or populations of commercial bioagents and the assessment of the genetic diversity of biomaterial from different manufacturers. Genetic analysis of *H. hebetor* populations show that all studied insect populations formed two distinct clusters. The studied geographical samples of insects from Belgorod and Chimkent belong to two genetically close *H. hebetor* populations. In the studied Russian biomaterial (Belgorod), we have traced the transfer of the genetic material of populations introduced from the regions of Central Asia. In this regard, one can characterize the relatively low quality of the three studied populations. Therefore, to improve the quality of bioagent population data, it is necessary to update them. At the same time, individuals of the Krasnodar population showed high levels of genetic diversity and DNA polymorphism. This, in turn, indicates a significant heterogeneity of the ectoparasite population.

The Krasnodar population released in the apple orchard showed its effectiveness at the level of 72.3%. Consequently, the application of *H. hebetor* in the orchard protection system against the codling moth and other lepidoptera pests is advantageous.

Apple orchards are heavily treated crops, and some sprayed insecticides are recognized to have toxic effects on non-target arthropods [31–33]. We have tested the sensitivity of *H. hebetor* and *H. axyridis* to biological and biorational preparations for the complex use of entomophagous species in integration with other apple orchard protection products. Lepidocid®, FermoVirin/YP®, Insegar®, Atabron®, Madex twin®, Insegar®, and some others proved to be compatible with these beneficial insects. At the same time, entomophagous species proved to have zero compatibility with the Decis Expert® pyrethroid preparation.

Aphids (Homoptera, Aphididae) are extremely damaging to agricultural plants. Traditional insecticides irregularly provide a positive result due to the rapid formation of resistance in many aphid species [34–36]. As practice shows, natural populations of aphidophagous predators are able to suppress aphids only by the end of June and the beginning of July, when their number noticeably increases. Therefore, the creation of reproducing reserves of aphidophagous species in early spring prevents the accelerated increase in

their populations' density. The introduction of laboratory-bred aphidophagous predators (ladybug larvae and the parasitoid *A. colemani*) into aphid foci led to a decrease in the pest population. At the same time, let us mention a significant economic effect. It is determined by a decrease in the release rates of entomophagous species, replenished by the reproduction and active migration of the introduced and natural populations of aphidophagous species. This is comparable to a natural biolaboratory cycle.

In the central part of Krasnodar Krai, and in many other regions, favorable conditions are being created in apple orchards for the development of pests with a large number of generations. These include, in particular, herbivorous mites [37–39], which have 2–12 generations per season. The release of laboratory populations of acariphagous predators (*A. andersoni* and *M. occidentalis*) into the tetranychid mite foci led to significant harm reduction. At the same time, the absence of chemical treatments allowed laboratory populations to develop steadily and accumulate their numbers. This, in turn, leads to a prolonged effect and a restoration of the mechanisms of natural biocenotic regulation.

5. Conclusions

1. We mass-bred the leaf-rolling race of the ectoparasitoid *H. hebetor* and released it at the Shcherbakov farm at a rate of 1200 individuals per hectare. The degree of fruit damage by the codling moth was 3.8%. The total parasitism of pest caterpillars in trapping belts reached up to 80%.

2. The sensitivity of entomophagous ectoparasitoid *H. hebetor* and the ladybug *H. axyridis* to biological preparations was studied. Under Madex twin®, Atabron®, and Koragen® on *H. axiridis*, the survival rates of aphidophagous adults on the fifth day were 97.3%, 89.6%, 81.9%, respectively. When exposed to Lepidocid® and Insegar®, 79.8% and 98.4% of cocoons of *H. hebetor* remained viable, respectively. The use of Atabron® and FermoVirin/YP® did not affect the emergence of *H. hebetor* from the treated cocoons (recovery was 100% in both cases).

3. The genetic diversity (Nei's index) within the geographic samples from Belgorod and Shimkent (Hs = 0.109) was 92% of the total genetic diversity (Ht = 0.119). These data suggest that the studied geographical samples of insects from Belgorod and Shimkent belong to two genetically close populations of *H. hebetor*.

The genetic variability within the Krasnodar and Stavropol samples was Hs = 0.186 of the total genetic variability Ht = 0.248. This indicates that the share of intrapopulation variability accounts for 75%, while the share of interpopulation variability is 25%.

4. The reservoirs with aphidophagous species helped to control the number of aphids during the formation of the leaves in the early spring, when the density of the natural population of Coccinellidae was low. The method efficiency was 82.8–88.6%.

5. The application of the acariphagous species *A. andersoni* and *M. occidentalis* promoted the effective control of herbivorous mites on an apple tree. The biological efficiency was at least 90%.

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