



## Application of innovative biological agents for sustainable corn farming

### Aplicación de agentes biológicos innovadores para el cultivo sostenible de maíz

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(recibido/received: 25-marzo-2023; aceptado/accepted: 05-mayo-2023)

#### ABSTRACT

Corn, being the most important crop globally, requires effective protection against pests while maintaining environmental sustainability. This study focuses on the development of environmentally friendly methods for biological control of major corn pests. The research investigates the combined use of pheromones, entomopathogenic preparations, and parasitic Hymenoptera, such as *Trichogramma evanescens* West and *Habrobracon hebetor* Say, to combat pests. The seasonal dynamics and abundance of cotton moth (*Helicoverpa armigera*) and click beetle (*Athous tauricus*) were monitored using pheromone traps. The number of corn moth (*Ostrinia nubilalis*) caterpillars was recorded, and mass breeding of the ectoparasite *Habrobracon hebetor* was conducted for biological control. The effectiveness of the integrated approach, combining entomophages and biological preparations, was evaluated. The study demonstrates satisfactory effectiveness in biological protection, with significant reductions in corn moth and cotton moth populations. These findings highlight the potential of environmentally friendly techniques for pest control in corn crops, contributing to sustainable agricultural practices.

**Keywords:** Corn; Environmental Sustainability; Entomophages; Biological Protection.

#### RESUMEN

El maíz, siendo el cultivo más importante a nivel mundial, requiere una protección eficaz contra las plagas manteniendo la sostenibilidad ambiental. Este estudio se centra en el desarrollo de métodos ecológicos para el control biológico de las principales plagas del maíz. La investigación investiga el uso combinado de feromonas, preparados entomopatógenos e himenópteros parásitos, como *Trichogramma evanescens* West y *Habrobracon hebetor* Say, para combatir plagas. La dinámica estacional y la abundancia de la polilla del algodón (*Helicoverpa armigera*) y el escarabajo clic (*Athous tauricus*) se monitorearon utilizando trampas de feromonas. Se registró el número de orugas de la polilla del maíz (*Ostrinia nubilalis*) y se llevó a cabo la crianza masiva del ectoparásito *Habrobracon hebetor* para el control biológico. Se evaluó la eficacia del enfoque integrado, que combina entomófagos y preparaciones biológicas. El estudio demuestra una eficacia satisfactoria en la protección biológica, con reducciones significativas en las poblaciones de polilla del maíz y polilla del algodón. Estos hallazgos resaltan el potencial de las técnicas ambientalmente amigables para el control de plagas en cultivos de maíz, contribuyendo a prácticas agrícolas sostenibles.

**Palabras claves:** Maíz; Sostenibilidad Del Medio Ambiente; Entomófagos; Protección biológica.

## 1. INTRODUCCIÓN

Annually, losses caused by corn pests on average amount to 10-20% of the crop, and with mass reproduction, grain damage can exceed 30%. Not only does the yield decrease but also the quality of grain deteriorates (Fitt & Wilson, 2000; Pogue, 2004; Khuzhamshukurov, 2016).

Corn is one of the most vulnerable crops to pests, of which the most damage is caused by the corn moth *Ostrinia nubilalis* L, cotton moth *Helicoverpa armigera* Hb, corn leaf aphid *Rhopalosiphum maidis* Fitch, and wireworms (Coleoptera, Elateridae) (Jalali et al., 2015; Chen et al., 2019; Cabrera Walsh et al., 2020; Costa et al., 2021). The corn moth *Os. nubilalis* L. is a pest of thick-stemmed crops. The main nature of the harm caused by it is damage to the core of stems and fibrovascular bundles, prevention of nutrient intake, which is accompanied by severe dehydration of the plant, increased fragility of stems, and, as a result, reduced productivity. Most of the time, moth caterpillars feed covertly, making moves inside plants. Their activity often leads to damage to the base of the cob, as a result of which the latter prematurely returns to an upright position. Caterpillars that have completed feeding overwinter inside plant residues. To a large extent, poor-quality sealing of plant residues, in which the bulk of the corn moth is concentrated for the next season, contributes to the development of the pest (Frolov & Malysh, 2004).

The cotton moth *H. armigera* Hb. is a multi-eating pest (polyphage). It damages at least 250 species of cultivated and weedy plants in different parts of its habitat. One of the most preferred plants is tomato *Lycopersicon lycopersicum* L. and corn *Zea mays* L. In the Krasnodar Territory, the cotton moth develops in three generations. The overwintered generation of the phytophagous first inhabits weeds, alfalfa, and subsequent generations inhabit tomato, soy, and corn. *H. armigera* can also cause great harm to tobacco plants (Bengtsson, Ahnstrom, Weibull, 2005; Fefelova & Frolov, 2007; Govorov, Zhiviykh, Proskuryakova, 2013; Agaseva et al., 2019).

A promising bioagent designed to reduce the chemical load on corn crops is the ectoparasite *Habrobracon hebetor* Say (Hymenoptera, Braconidae), known as a parasite of more than 60 species of lepidopteran pests (Agaseva & Ismailov, 2016; Ismailov et al., 2017; Agasyeva et al., 2021). *Habrobracon* females can penetrate various holes, cracks, and other places where phytophagous caterpillars settle, in particular the corn stem moth and the cotton moth, which feed inside the stems, fruits, and ears. Against the corn leaf aphid (*R. maidis*), effective bioagents are *Aphidius colemani* Vier. and *Harmonia axyridis* Pallas.

The study aimed to develop a system of biological protection of corn from major pests based on the use of entomophages, entomopathogens, and natural biocenotic regulation.

## 2. METHODOLOGY

The study was carried out at the experimental site of the Federal State Budgetary Scientific Institution "Federal Research Center of Biological Plant Protection" (FSBSI FRCBPP) (Krasnodar) with an area of 1 ha, sown with a hybrid of early ripening corn (Krasnodarsky 291 AMV), as well as on the farm of Agronova LLC (Labinsky district of Krasnodar Territory), with an area of 3 ha, certified according to an organic standard, on a hybrid Krasnodarsky 455 AMV of late ripening in 2018-2019.

The seasonal dynamics of flight and the abundance of *H. armigera* and the click beetle *A. tauricus* were determined by trapping males with pheromone traps. For this purpose, on experimental plots of corn with an area of 50 m<sup>2</sup> in advance (10-15 days before the start of the seasonal flight), three Atrakon A traps with a cotton moth pheromone and Estren traps with an *A. tauricus* pheromone were installed. The records were carried out twice a week during the entire growing season.

The number of caterpillars of the cotton moth *H. armigera* was recorded before the formation of cobs on 20 plants at different points of the field, and after the formation of cobs on 20 cobs in four repetitions.

The count of the number of caterpillars of *Os. nubilalis* was carried out on 15 plants on different plots in 6-7-fold repetition. At the end of the growing season, the stems were cut at the soil level, split lengthwise to the base with a knife and we counted the caterpillars of the corn moth in the stems before harvesting.

Mass breeding of ectoparasite *H. hebetor* for biological control of corn moth and the cotton moth was carried out using caterpillars of the wax moth (*Galleria mellonella* L.) in the middle instars grown on an artificial nutrient medium (ANM) as a host insect (Shirinyan, Ismailov, Kvasenkov, 2003). For this, 100 g of ANM and 7-10 cocoons of *G. mellonella* were placed in clean glass jars with a capacity of 1 l (before the butterflies flew out of them). The jars were covered with glass lids and placed in a dark thermostat with a temperature of 28-30°C and air humidity of 70-75%. The butterflies that had flown out laid eggs directly on the environment, of which after 12-15 days, caterpillars were hatched and immediately introduced into the environment. In the jars, the caterpillars developed up to the 3rd age. Then the contents of the jars were divided into two portions, which were placed (one at a time) in new jars with a capacity of 1 l, pre-filled with a medium layer of 6-7 cm. This amount of medium would be enough until the caterpillars were fully grown.

Wax moth caterpillars of older instars (100-400 caterpillars) were placed on corrugated paper in glass jars with a capacity of 1-3 l to infect them with *Habrobracon*. 50-70 entomophages were put into each jar to paralyze the caterpillars and lay eggs on them. After 10-14 days after the flight of adult parasites, they were put in glass jars with a capacity of 0.25-0.5 l and released to corn crops during the appearance of corn moth and cotton moth caterpillars in the middle instars at the rate of 1-2 thousand individuals per 1 ha.

The following biological preparations were also used to protect corn from pests: Vertimek, CE (18 g/labamectin, Syngenta, Switzerland), Lepidocide, P (*Bacillus thuringiensis* var. *Kurstaki*, BA-3000 EA/mg, with a titer of at least 60 billion spores/g, Sibbiopharm, Russia), and Helicovex, CK (cotton moth nuclear polyhedrosis virus,  $7.5 \times 10^{12}$  polyhedra/l, Andermatt Biocontrol AG, Switzerland).

The work was carried out using the material and technical base of the USI (unique scientific installation) "Technological line for mass breeding of insect entomophages" (Unique Scientific Facility, n.d.), as well as the objects of the biological resource collection (BRC) "State Collection of entomoacariphages and microorganisms" of the Federal Scientific Center for Biological Plant Protection (State Collection of entomoacariphages and microorganisms, n.d.).

### 3. RESULTS AND DISCUSSION

In 2018-2019, the main phytophages were identified in corn crops, which include the corn moth *O. nubilalis* L. and cotton moth *H. armigera* Hb., turnip moth (*Agrotis segetum* Schiff), heart and dart moth (*Agrotis exclamationis* L.), silver moth (*Autographa gamma* L), and small mottled willow moth (*Laphygma exigua* Hb.). Aphids (*Hemiptera*, *Aphididae*) caused significant damage. The larvae of click beetles damaged the seeds and seedlings of plants, which often leads to the thinning of corn crops. The click beetle *Agriotes tauricus* Heyd. was identified on experimental plots.

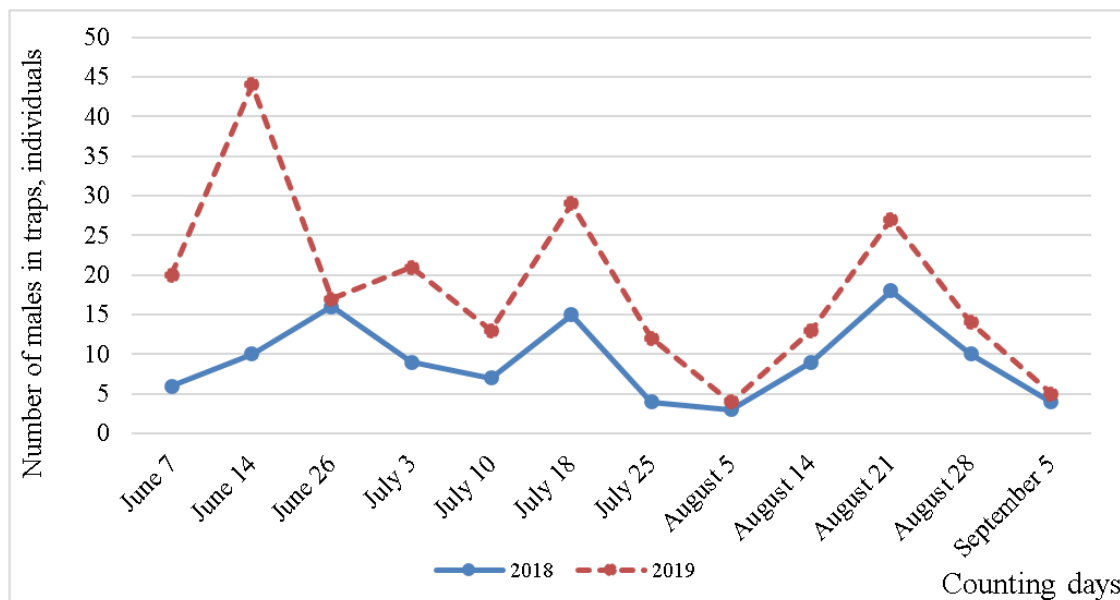
Corn crops were located next to barley crops. As a result, at the end of the growing season of barley, there was a migration of cereal leaf beetle (*Oulema melanopus* L.) to the experimental plots of corn. The number of cereal leaf beetle was 3-5 individuals/plant. To control the cereal leaf beetle, treatment with the biorational preparation Vertimek, CE was carried out at a consumption rate of 1.2 l/ha. The accounting carried out after processing showed that the number of pests had decreased significantly and amounted to 0.5 individuals/plant.

In the second ten-day period of May, glue traps with cotton moth pheromone were hung out on experimental plots.

A characteristic feature of the phenological development of the cotton moth in 2018 and 2019 is a very high number of the overwintered generation of the pest [20], which is confirmed by the mass flight of males in the first and second ten-day periods of June from 15 to 35 individuals/trap for 7-8 days (Figure 1).

As can be seen from the data presented in Figure 1 (2018), the number of males of the overwintered generation caught by pheromone traps began to decrease from June 14, and the flight of butterflies practically stopped. The flight of the first summer generation of the cotton moth began on July 5 and was continuous, it reached its maximum in the period from July 5 to July 25, which averaged 10-15 individuals/trap, and then slightly decreased to 3-4 males per trap on July 5, but after replacing pheromone dispensers, the flight again increased to 10 individuals/trap in a week.

In 2019, there were three distinct generations of the cotton moth with flight peaks in June, July, and August (Figure 1).



\*Source: compiled by authors

Figure 1. Dynamics of the flight of males of the cotton moth *H. armigera* (FGBNU FNTsBZR, 2018, 2019)

To determine the number of wireworms (larvae of click beetles), traps with a synthetic pheromone of the click beetle *Athous tauricus* Candeze were set at the beginning of the second decade of June (Figure 2).



\*Source: compiled by authors

Figure 2. Pheromone trap for catching a male of the click beetle *A. tauricus*

It was found that the flight of the *A. tauricus* males into pheromone traps began in the third decade of June and amounted to 19.6 individuals per trap on average per week, and the maximum peak of catching 160 individuals/trap was registered on July 17. According to the number of beetles captured during the seasonal flight of the *A. tauricus* click beetle (June 21 – August 7, 2019).

The main kind of harm caused by the corn moth *Os. nubilalis* is damage to the core of the stems. Most of the time, moth caterpillars feed covertly, making moves inside plants, most often stems, where they penetrate in the first or third instars. A Trichogramma *Trichogramma evanescens* West was released twice on the ovipositors of the corn moth and cotton moth, at the rate of 3 g of infected eggs of *Sitotroga cerealella* Olivier eggs (150-200 thousand Trichogramma individuals) per 1 ha. Then, after the hatching of the caterpillars of the corn moth and the cotton moth, treatments with Lepidocide, P (3 kg/ha) + Helicovex (0.2 l/ha) were carried out, and when the caterpillars of the middle instars of these pests appeared, two releases of the Habbrobracon ectoparasite were made at the rate of 1,500-2,000 individuals/ha (Table 1).

Decisions on carrying out protective measures were made strictly according to the results of monitoring, considering the number of pests determined by catching males with pheromone traps, visual observations, the sweep-net method, as well as establishing the entomophage effectiveness levels (EEL) based on the ratio of pests and their entomophages.

The results presented in Table 1 show that the biological effectiveness of the biological protection of corn from the corn moth was 84% on an early hybrid and 94% on a late one, and the effectiveness of protection against cotton moth equaled 82.2-77.8%, respectively.

Table 1 – Biological effectiveness of comprehensive protection of corn from major pests

Hybrids	Type of insect	Number of insects, individuals per 1 plant, per accounting dates							Biological effectiveness, %
		3.07	10.07	19.07	24.07	2.08	13.08	total	
Krasnodarsky hybrid 291	Corn moth	0	0	0.3	0	0	0	0.3	84.1
AMV (early maturation)	Cotton moth	0	0.12	0.08	0	0.3	0.7	1.2	82.2
Krasnodarsky hybrid 455	Corn moth	0	0	0.07	0	0	0	0.07	94.0



MV (late maturation)	Cotton moth	0	0.06	0.1	0	0.3	0.5	0.96	77.8
Control	Corn moth	0.38	0.18	0.6	0	0	0	1.16	-
	Cotton moth	0	0.51	0.7	0.9	1.0	1.2	4.31	-

\*Source: compiled by authors

#### 4. CONCLUSIONES

Thus, with the help of pheromone traps and other monitoring methods, as a result of a field assessment of the biological control system of the main corn pests, the satisfactory effectiveness of mutually reinforcing techniques based on the use of *Trichogramma* and *Habrobracon* entomophages and biological preparations based on entomopathogenic bacteria and viruses has been established. The species composition of corn pests has been clarified, and the dynamics of flight and the number of dominant pests, namely the cotton moth *H. armigera* and the corn moth *Os. nubilalis* were determined, and the optimal timing of protective measures was established (release of entomophages (*Trichogramma* and *Habrobracon*), treatment with biological preparations).

The developed techniques can be effectively used for biological control of the main corn pests, both separately and in combination with entomophages within the framework of organic farming technologies.

#### ACKNOWLEDGMENTS

The study was carried out according to the State task of the Ministry of Science and Higher Education of the Russian Federation within the framework of research on topic No. FGRN-2022-0003.

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