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Method for disrupting the reproductive pheromone bond of the cotton bollworm on sunflower crops

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Abstract

Background: Synthetic analogs of sexual pheromones provide an effective method of biorational control of the number of phytophagous insects based on the directed disruption of reproductive functions in populations of the target pest on the protected crop without affecting the integrity of the agrocenosis. The work aims to determine the biological effectiveness of methods of mass trapping and disorientation of the cotton bollworm (*Helicoverpa armigera*) to protect sunflower (Helianthus annuus) crops.

Methods: The experiments were carried out in 2022-2023 in the Central Zone of the Krasnodar Territory, Russia in the stationary scientific crop rotation at the Federal Research Center for Biological Plant Protection, Krasnodar (2.5 ha) and the Kononenko farm, Novovelichkovskaya village, Dinsky district (10 ha). The biological effectiveness of elimination and disorientation methods was judged by the degree of damage to plants and buds. The disorientation effect was calculated by the number of males captured at the disorientation sites compared to the control sites.

Result: The decrease in males' search activity (disorientation effect) reached 85.3%. The damage to buds by the pest in this variant was 2.0% compared to 10.8% in the control group. In the mass trapping variant, it equaled 1.5-3.0 and 7.5%, respectively, indicating the prospects of including these techniques in the *H. armigera* biorational control system.

Conclusion: Our findings could aid future research on insect chemical communication to develop effective biological control methods.

Introduction

Sunflower (*Helianthus annuus*) is the most important oilseed crop in global and Russian crop production [1,2]. The main sunflower pests are the Lepidoptera order (mainly the Pyralidae and Noctuidae families) [3]. Lepidopteran phytophages damage plants at almost all stages of development, from seedlings to bud maturation, which without effective protective measures can lead to a decrease in yield by up to 40% [4-7]. When choosing sunflower protection products, most agricultural producers continue to use traditional chemical pesticides[8], which, in addition to negative effects on beneficial species and the environment[9,10], lead to the formation of resistant pest insect populations. Hence, there is a need to use alternative biologically safe plant protection products [11,12].

Biologically active substances of biogenic origin are promising for regulating the number of harmful Lepidoptera species[13]. The most popular of these substances are synthetic sex pheromones [14], which have been widely used since the 1950s when the structure of the pheromone of the domestic silk moth (*Bombyx mori)*, was first identified and its synthetic analog, bombykol, was obtained. They are used globally for phytophage monitoring [14]. In addition to monitoring, pheromone materials are used to regulate the number of pests by methods of elimination, mass trapping of males, disorientation, and auto sterilization [14,15].

Mass trapping (elimination) of Lepidoptera pests is carried out using various traps (pheromone, light, LED, etc.) [16-18]. However, as several studies have shown, pheromone traps are selective and ensure the capture of males of only the target species [19,20].

Disorientation as an independently developed method of plant protection is based on a disturbance of the olfactory relationship between the sexes [21,22]. The effect of male disorientation is achieved by a high concentration of pheromone in the surface air layer of the protected crop, many times exceeding the number of molecules of signaling substances produced by female insects. The high background concentration of the pheromone results in the inability of the male to detect a small amount of the substance secreted by the female. The constant stimulating effect of the pheromone accelerates the death of males and leads to a decrease or complete cessation of the secretion of signaling substances by females. This, considering the pre-adaptation of chemical receptors and the central nervous system, leads to a disturbance of reproductive functions in insect populations and makes this method promising [21-23]. Only in recent years, disorientation has been successfully applied in the USA, Japan, Australia, Russia, and the EU against the codling moth (*Cydia pomonella*) on more than 200 thousand ha, the oriental fruit moth (*Grapholita molesta*) on more than 50 thousand ha, the European grapevine moth (*Lobesia botrana*) on more than 60 thousand ha, and the vine moth (*Eupoecilia ambiguella*) on more than 45 thousand ha [23]. Experiments on the disorientation of the cotton bollworm (*Helicoverpa armigera)* have so far been carried out in limited areas and not always successfully [21,22]. This determines the need for research in this direction.

The study aims to evaluate pheromone material methods to regulate the number of Lepidoptera pests on sunflowers.

Methods

The study was carried out in the Central zone of the Krasnodar Territory, Russia on sunflower crops on 2.5 ha at the Federal Research Center for Biological Plant Protection (FRCBPP), Krasnodar and on 10 ha at the Kononenko farm, Novovelichkovskaya village, Dinsky district in 2022-2023. The experiment area is characterized by the following climate: the average annual temperature is 10.4-10.6oC, the frost-free period is 194-195 days, the annual precipitation is 570-583 mm, and the moisture coefficient is 0.3-0.4.

The synthesis of the cotton bollworm pheromone, consisting of a mixture of cis-11-hexadecenal and cis-9-hexadecenal in a ratio of 10:1, was carried out in the Laboratory of Chemical Communication and Mass Breeding of Insects at the FRCBPP. The pheromone formulations were manufactured using specially designed technological equipment at the FRCBPP which allows to apply the active substance to the inside of a medical rubber tube. Glue traps for monitoring, mass trapping of males, and controlling the effect of disorientation of the cotton bollworm (Atrakon type) were manufactured using the FRCBPP technological equipment. The experiments were carried out on the medium-ripened sunflower of Umnik variety (selection by the All-Russian Research Institute of Oil Crops) at the FRCBPP and the Master variety (selection by the All-Russian Research Institute of Oil Crops) at the Kononenko farm.

To determine the swarming dynamics and the number of pests, we placed 1 pheromone trap/1-3 ha on experimental plots. The attracted males were counted once a week. In experiments on mass capture of insects (elimination), we placed 5-10 traps/1 ha. The experiments were carried out on a plot at the Kononenko farm, and the control plot was a site without pheromone traps. The distance between the variants of the experiment was 200 m.

The experiments on disorientation were carried out on sunflower sowing in a stationary 8-field scientific crop rotation at the FRCBPP. To carry out disorientation, we placed special rubber rings with an initial pheromone rate of 5 mg/1 ring in the amount of 400 rings/1 ha at a height of 0.8-1 m from the soil surface, evenly distributing them over the experimental plot. To account for the effect of disorientation, five pheromone traps were installed at the experimental and control plots, and the males in the traps were regularly counted (with an interval of 4-5 days). The distance between the plots was 200 m.

The disorientation effect was calculated by the number of males captured in the experimental plot compared to the control one.

The biological effectiveness of elimination and disorientation was judged by the degree of damage to plants and sunflower buds. The species composition and population dynamics of other Lepidoptera species in sunflower crops were assessed using pheromone traps manufactured by the FRCBPP.

The results were processed according to generally accepted statistical analysis methods using the Statistica v.12.6 software (StatSoft, Inc., USA). The tables show the mean (*M*) and standard errors of the mean (±SEM).

Results

The Lepidoptera entomological fauna on sunflowers was mainly represented by species of the Noctuidae and Pyralidae families. From the Noctuidae family, we found the cotton bollworm *H. armigera*, turnip moth *Agrotis segetum*, heart and dart *Agrotis exclamationis*, and silver Y *Autographa gamma*. The Pyralidae were more often represented by the beet webworm *Loxostege sticticalis* butterflies. During two years of research, we found that the cotton bollworm was the dominant species; the number of males caught averaged 12 specimens/trap in one week, while the number of males of other species was significantly lower (on average, no more than 2.5-5.0 specimens/trap in one week) (Figure 1). Thus, we tested methods of mass trapping and disorientation of males with the help of pheromones aimed at cotton bollworms. It should be noted that recently, *H. armigera* numbers and harmfulness have significantly increased [25].

In the Central zone of the Krasnodar Territory, the cotton bollworm develops in three generations. Figure 1 demonstrates the dynamic of insects swarming at Kononeko farm, measured every 7 days. During the analyzed period, the number of the first summer generation of *H. armigera* was significantly higher than the economic injury level (EIL) (8 specimens/trap per week) and amounted to 24.5 specimens/trap per week in July. The cotton bollworm swarming season began in 2020-2021 in the second decade of May and lasted almost until September. When studying the circadian rhythm of the pes's sexual activity, we found that the maximum swarming of males in the pheromone traps occurred from 7-8 pm to 4-5 am, which is consistent with the available literature data [16].

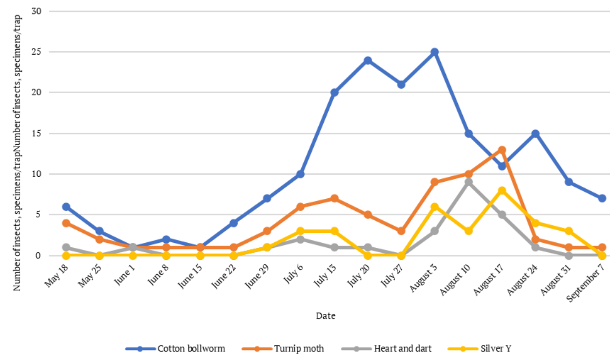

Figure 1: Dynamics of the Lepidoptera pests swarming at Kononenko farm.

We carried out the mass capture of *H. armigera* males during the swarming of the most numerous first summer generation of the pest. Test results of mass trapping of *H. armigera* males can be seen in Table 1. The greatest efficiency of the elimination method was achieved when installing 10 traps/ha; the damage to buds reached 1.5%. When using 5 traps/ha, the damage to buds was 3.0%. In the control variant, the damage reached 7.5%.

Accounting date	Attracted males, specimens/trap		
	10 traps/ha	5 traps/ha	1 trap/ha (control)
12.07	13.8±2.3	10.5±2.5	13.0±4.0
19.07	12.6±1.8	12.8±3.7	14.0±2.9
26.07	14.4±3.1	13.2±1.6	14.0±4.9
04.08	10.2+2.7	11.6±3.2	12.0±2.7
12.08	8.3±1.7	8.5± 2.1	7.0±2.0
20.08	5.6±1.5	5.4± 1.7	5.0±1.7
27.08	3.2±1.2	2.9±1.0	3.0±1.3
Total per 1 trap, specimens	68.1±7.2	64.9±6.3	68.0±5.6
Total, specimens	681.0±12.4	324.5±10.4	68.0±5.6
Damaged buds, %	1.5	3.0	7.5

Table 1: Test results of mass trapping of *H. armigera* males.

Discussion

One of the methods of disrupting pheromone communication used against many insect species is disorientation. The disorientation mechanism leads to the adaptation of male chemical receptors, as a result of which the search for females and mating of insects within the population are disrupted.

The field experiments on breaking *H. armigera* pheromone bond showed that saturation of the surface air layer with synthetic pheromone vapors effectively suppressed the ethological reactions of males when searching for the source of the sexual pheromone. The disorientation effect depends on the rate of emission of synthetic pheromones; materials used as dispensers play an important role [26]. Special rubber was previously selected for them, providing prolonged pheromone emission for 2 months after the dispensers were placed. Table 2 demonstrates the number or attracted males in the disorientation area opposed to the control variant.

A high disorientation effect on the cotton bollworm was achieved in the experiments (Table 2). At a consumption rate of 2 g/ha (400 pheromone dispensers/ha), the effect amounted to 85.3%. The damage to buds was 2.0% in the experimental and 7.8% in the control variant.

Accounting date	Attracted males, specimens/trap	
	Disorientation area	Control
01.07	3.8±1.3	10.0±3.2
08.07	3.6±1.0	23.6±5.3
13.07	3.5±1.9	25.0±4.8
15.07	3.2±2.6	23.0±3.9
20.07	4.3±1.8	26.0±4.5
03.08	3.6±2.1	18.0±3.1
12.08	3.0±1.9	12.0±2.6
17.08	2.0±1.5	18.0±3.7
23.08	2.0±3.1	11.0±2.5
31.08	1.0±1.3	7.0±2.8
Total, specimens	30.0±4.7	203.6±10.3
Disorientation effect,%	85.3	-
Damage to buds, %	2.0	10.8

Table 2: The effectiveness of disorientation of *H. armigera.*

Thus, the obtained results demonstrate the sufficient effectiveness of sunflower protection methods from cotton bollworms based on the disruption of sexual chemical communication of insects and can be recommended for testing in *H. armigera* biological control systems in organic farming.

Based on field tests of synthetic sex pheromones for biorational control of *H. armigera* numbers on sunflower crops, we established the fundamental possibility of using elimination and disorientation of males in combination with other biological and biorational plant protection products. The effectiveness of disorientation was 85.3%, and the damage to sunflower buds did not exceed 2.0% compared with 10.8% in the control variant. In experiments on mass capture (elimination) of males, the damage to buds was 1.5-3.0% compared with 7.5% in the control variant.

Our results can be useful in further studies of intraspecific and interspecific chemical communication of insects to develop effective methods of biological control of harmful species.

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Author Contributions

V.Ya. Ismailov conceptualized the research project and designed the methodology. A.A. Komantsev conducted the data analysis and interpretation. V.I. Borodin was responsible for data curation, investigation and reviewing and editing of the paper.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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