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METHODOLOGICAL AND TECHNOLOGICAL METHODS FOR APPLICATION OF SEXUAL PHEROMONES AGAINST *GRAPHOLITHA FUNRBRANA* Tr.

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Key words*: sexual pheromone, grapholitha funebrana, female, male, trapping, disorientation*

INTRODUCTION

The application of sexual pheromones in embedded systems of plant protection requires extensive investigations in assessing the biological specificity of the behavior reaction in the imago phase of target-insect species. Therefore, considering the behavior of the species *Grapholitha funebrana Tr.*, this is an important pest of plum culture and represents a particular interest from a practical standpoint. For the estimation of biological activity and specificity of the synthetic sexual pheromones the most appropriate is the method of assessing the terms in the field, based on the attraction of males in traps. One of the main elements of technologically consists in assessing the biological effectiveness of sex pheromone composition and the giver.

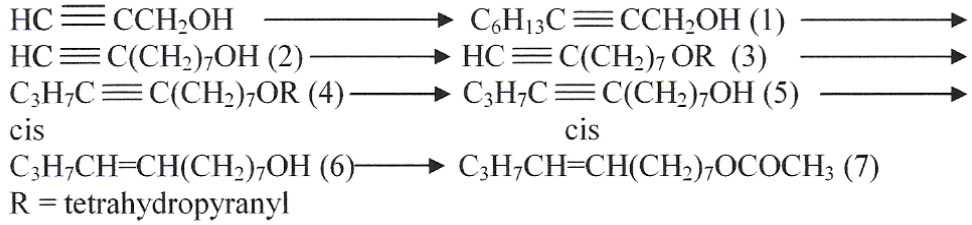
For the estimation of biological activity and specificity of the synthetic sexual pheromones the method of their assessing in terms of the fields the more appropriate, which is based on the attraction of males in the traps provided with the corresponding trolls. One of the main elements from the technological point of view consists in assessing the biological effectiveness of sex pheromone composition and the giver.

In the methods of synthesis of sexual pheromone of the species *Grapholitha funebrana* have been used the substances, which are quite expensive and toxic. To liquidate these shortcomings we have proposed to modify the scheme of synthesis of the main component of the sex pheromone of phytophagous *G. funebrana* – "Z8-12AC".

The aim of researches was to optimize the composition of synthetic sexual pheromone of the pest *Grapholitha funebrana*, and to develop methodological and technological processes regulating the population density.

MATERIAL AND METHODS

The method of synthesis of the main sexual pheromone component of the species *G. funebrana* – "Z8-12AC" was performed based on a modified scheme developed by us:



Intermediate and final substances were purified by vacuum distillation and silica gel column purification. Their purity was identified by gas-liquid chromatography at Chrome 5 and HP 5890 chromatographs.

Pheromone traps for assessing the effectiveness of the method for mass capture of males were placed in the recital by 12/ha. They were replaced every 14 days with adhesive supplements and once a generation – dispensator of pheromones. Evidence of males captured in traps was performed every 7th day.

As a material for making the dispensators of pheromones there have been used the synthetic rope with an inner layer of thin un wring polymer fibers. The active substance of the pheromone was applied to segments of string by placing a pheromone in hexane solution. The length of string segments was 10 cm, and the amount of pheromone applied was read 1 mg/cm. To prove the rapid evaporation of pheromone out of dispensers were covered with a layer of polyvinyl chloride by dipping them for 1 minute in a solution of 8% of tetrahydrofuran. The dispensators contained for 10 mg of pheromone (Figure 1).

Fig. 1. Showing of pheromone dispensators for male's disorientation testing method *Grapholitha funebrana* pest, in terms of production.

The composition of the sexual pheromone [Z8-12Ac97% + E8-12Ac3%] (10%) + H-12AC (90%).The method of disorientation of males was tested under production. Plum orchard area constituted 5.4 ha. Dispensators were displayed in the recital by one per tree (500/ha) or 5.0 g pheromone/ha /generation. The biological efficacy of the method of disorientation was assessed by two criteria: 1. through appreciation of the locking rate of the reaction of males to pheromone traps; and 2. through appreciation of plum attacked by larvae. The record was made during the development of two generations with an interval of 7 days.

The results were subjected to mathematical analysis in accordance with the program package Microsoft Excel.

RESULTS AND DISCUSSIONS

Optimization of pheromone composition: Throughout the duration four compositions were subjected to testing pheromone of the pest *G. funebrana* in which the rate of the minor component gradually was increased "E8-12AC" and reduced the rate of the main component "Z8-12AC" (Table 1).

Tests under field conditions have demonstrated that at the same time with the increase of the rate of the minor component "E8-12AC" in pheromone composition is substantially reduced the biological effectiveness of it. Based on the results it was found that the higher rate of minor components in pheromone composition, the smaller the number of males who have responded to these traps. Thus it was found that in the composition of the sexual pheromone of the species *G. funebrana* the rate of the minor component "E8-12Ac" is necessary not to exceed the limit of 3.0-4.0%.

Further investigations have undergone testing of other seven pheromone compositions in which the main component was substituted – "Z8-12Ac" with the minor component –"H-12Ac".The biological efficacy was appreciated by the number of males which have reacted to traps provided with appropriate pheromone compositions (Table 2).

Table 1. Sexual reaction manifested by male of *Grapholitha funebrana* species to pheromone traps depending on the rate of the minor component "E8-12AC"

Variants	I generation			II generation		
	The number of males	Deviation from the witness	Group	The number of males	Deviation from the witness	Group
Witness Z8–12Ac(97%) + E8–12Ac(3%)	240,0	-	-	394,0	-	-
Z8–12Ac(95%) + E8–12Ac(5%)	182,0	- 58,0	III	206,0	- 188,0	III
Z8–12Ac(90%) +E8–12Ac(10%)	76,0	- 164,0	III	192,0	- 202,0	III
Z8–12Ac(85%) + E8–12Ac(15%)	30,0	- 210,0	III	100,0	- 294,0	III
		$DEM_{0,05} = 44,0$			$DEM_{0,05} = 47,0$	

Table 2.Sexual reaction manifested by males of the species *Grapholitha funebrana* to traps equipped with different compositions of synthetic sexual pheromone

Variants	I generation			II generation		
	The number of males	Deviation from the witness	Group	The number of males	Deviation from the witness	Group
Witness Z8–12Ac(97%) + E8–12Ac(3%)	197,4	-	-	97,0	-	-
E8–12Ac(3%) +C-12Ac(97%)	0	- 197,4	III	0	- 97,0	III
[Z8–12Ac97% + E8–12Ac3%](65%) + H-12Ac(35%)	257,4	+ 59,6	I	124,0	+ 27,0	II
[Z8–12Ac97% + +E8–12Ac3%](50%) + H-12Ac(50%)	247,8	+ 50,4	II	67,3	- 29,7	II
[Z8–12Ac97% + E8–12Ac3%](35%) + H-12Ac(65%)	205,4	+ 8,0	II	65,5	- 31,5	II
[Z8–12Ac97% + E8–12Ac3%](25%) + H-12Ac(75%)	202,4	+ 5,0	II	84,0	- 13,0	II
[Z8–12Ac97% + E8–12Ac3%](10%) + H-12Ac(90%)	250,0	+ 52,6	I	117,0	+ 20,0	II
		$DEM_{0,05}$ = 51,8			$DEM_{0,05}$ = 57,8	

The analysis of the results showed that in the absence of the main component in the pheromone composition (version II) was caused total blockage of the sexual response of males of the species *G. funebrana.* Testing the following pheromone composition revealed the time that by substituting the main component – "Z8-12Ac" with the minor – "H-12Ac" (variants III-VI) was not due to reduced male sexual response. Thus it was found that a gradual replacement of the main component – "Z8-12Ac", which is very expensive, with the minor – "H-12Ac" - less expensive, do not cause reduction of the male sexual response of these species compared with the composition of standard synthetic sexual pheromone – "Z8-12Ac (97%) + E8-12Ac (3%)". Simultaneously, the results allowed the selection of an effective pheromone composition and economically cost-effective. Taking into account the results obtained during the tests, for further investigations there have been selected the following pheromone composition – "[Z8-12Ac 97% + E8 – 12Ac 3%] (10%) + H-12Ac (90%)".

Thus, it was experimentally demonstrated that pheromone composition developed in the first place – "[Z8-12Ac97% + E8-12Ac 3%] (10%) + H-12Ac (90%)" can be recommended for application because it is more efficient and economically cost effective than the standard pheromone composition – "Z8-12Ac (97%) + E8-12Ac (3%)", applied till the present.

Assessment of seasonal sexual cycle: As allure, in the traps was applied the composition of the pheromone "[Z8-12Ac 97% + E8-12Ac3%] (10%) + H-12Ac (90%)". The results showed that the species *G. funebrana* in the climatic conditions of the Republic of Moldova is developing in two generations. Thus it was found that the first generation begins its work at the end of the third decade of April, extending until the first decade of June. The peak of the sexual activity in the first generation was fixed at the end of the second decade and the beginning of the third decade of May. The sexual activity of second generation extends over a long period of time and is within the first decade of July and the first decade of September. The peak of sexual activity of males in the second generation was recorded at the end of the first decade of July to the end of the decade three of July (Fig. 2).

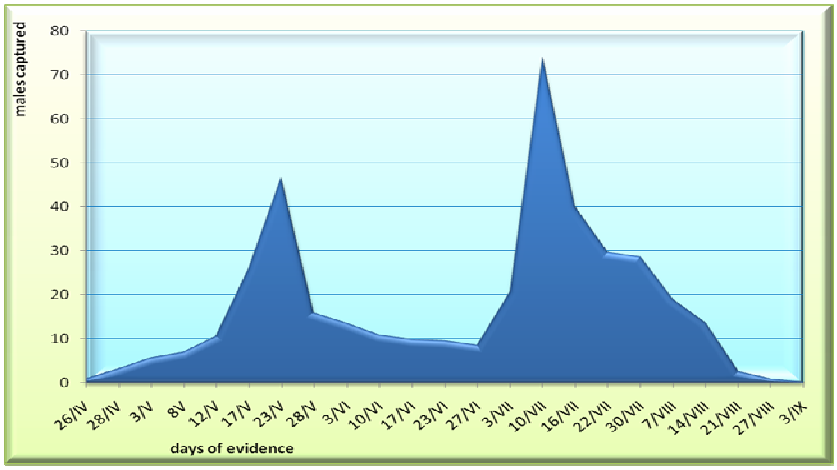


Fig. 2. Seasonal sexual cycle of the species *Grapholitha funebrana* under agricultural clime of the Republic of Moldova (2013)

Thus from making multiple investigations demonstrated that seasonal sexual cycle of phytophagous species *G. funebrana* extends over a period of about 120 days (late April to early September). The results can serve as a practical application of the various means of regulating population density of the given phytophagous species.

Given the characteristics of the sexual pheromone was considered as an aim the prove of management efficiency of species *G. funebrana* by different manner of their application. Such tests have been subjected to two methods: 1. Mass capture of males; and 2. Disorientation of males.

Mass capture method of *Grapholitha funebrana* males: The essence of the capture method is to eliminate out of the population of the species of the target insects of a major number of active males. Removing the active males influence on increasing the number of females non- coupled. Non-coupled females will not lay fertile eggs and thus the density of the target herbivore populations will be reduced.

Testing the method for mass capture of male *G. funebrana* was preceded first by determining the migratory capacity of males, and secondly - by assessment of range of action of the pheromone traps.

During testing it was found, that the experimental orchard, *G. funebrana* population density in the first generation was average (49.0 males/trap/7 days). Second generation population density was high (101.3 males/trap/7 days). In the range of the distance of 1 km from the experimental orchard were no other plum orchards. To assess the migratory capacity of males, pheromone traps were placed under the prevailing direction of the wind at a distance of about 25.0, 50.0 and 100 meters from the plum orchard. The number of males who responded to pheromone traps was compared with the number of those who responded to traps located inside plum orchard.

The analysis of the results showed that the number of males who responded to pheromone traps located outside the plum orchard was much lower than those that reacted to the traps located inside the orchard. To the pheromone traps located at a distance of 25.0 m from the orchard of plum, were reacted with 85.1% less males as traps located inside the plum orchard. To the traps placed at a distance of 50 and 100 m from the plum orchard, were attracted an even smaller number of males (8.2 to 4.2%). Thus it was shown that *G. funebrana* is not among the major species of migratory properties. This conclusion presents an important practical interest because it was found experimentally that it is unlikely the penetration of insects belonging to other populations in the appropriate plum orchard (Table 3).

Further investigations have been warned to determine the optimum height at which imago *G. funebrana* prefer to carry out the active mode of life - that is in the bottom, middle or top of the crown of plum trees. As a criterion to determine the optimal height of imago carrying the mode of life, have served the number of males captured in pheromone traps at appropriate heights. Traps were placed in the crown of the trees at the bottom - 1.0 m from the ground surface, medium - 2.0 m, and top - 3,0 m. The number of males captured was compared with those in traps placed at height of 2.0 m (as a witness). The results obtained are shown in Table 4.

Table 3. Rate of males of *Grapholitha funebrana* species attracted by the pheromone traps depending on their location away from the plum orchard

Variants	I generation			II generation		
	The number of males in a trap	Deviation from the witness	Group	The number of males in a trap	Deviation from the witness	Group
Witness	49,0	-	-	101,3	-	-
25,0 m from the orchard	7,3	- 41,7	III	31,0	- 70,3	III
50,0 m from the orchard	4,0	- 45,0	III	23,0	- 78,3	III
100,0 m from the orchard	2,0	- 47,0	III	17,0	- 84,3	III
		$DEM_{0,05} = 10,1$			$DEM_{0,05} = 26,8$	

Table 4. Rate of phytophagous *Grapholitha funebrana* species males attracted to pheromone traps depending on their height of location in the crown of the plum trees

Variants	I generation			II generation		
	The number of males in a trap	Deviation from the witness	Group	The number of males in a trap	Deviation from the witness	Group
Witness (traps located to the ground 2,0 m from the ground)	32,8	-	-	23,5	-	-
1,0 m from the ground	26,2	- 6,6	II	15,5	- 8,0	II
3,0 m from the ground	33,4	+ 0,6	II	20,5	- 30,0	II
		$DEM_{0,05} = 7,2$			$DEM_{0,05} = 13,6$	

Analysis of the results showed that for the species *G. funebrana* is not peculiar to a particular operating height. All along we believe that the optimum height of pheromone trapping for mass capture method (in practical terms) is 2.0 m above the ground.

Or investigated further with the aim of assessing the optimum number of pheromone traps needed to capture active males of the species *G. funebrana*. Three variants were fitted with a one hectare area, which were placed by 9, 18, and 27 pheromone traps. As a result was shown that placing of pheromone traps by 18 and 27 pheromone traps, not influencing essentially on biological efficiency compared to that obtained by placing the 9 traps per 1 ha. Therefore, in subsequent tests of the method of capture mass species *G. funebrana* males were placed by 10 pheromone traps per 1 ha.

Tests carried out were aimed to assess the biological effectiveness of the method for mass capture of males over several consecutive years in one and the same plum orchard. It is shown that the method of mass capture of male *G. funebrana* over three years in one and the same orchard leads to increase of biological efficiency. Given factor was confirmed by an essential reduction of population density on the sector subjected to testing of the method for mass capture of males, and therefore the rate of the attacked plums by larvae was reduced to a minimum. Thus, it was experimentally shown that by applying the mass capture of males is possible to reduce the harmfulness of the population of the species *G. funebrana* and get a green plum fruit (Table 5).

Method of disorientation of males *Grapholitha funebrana*: The principle of the method of disorientation action is as simple as that and sophisticated as it affects sexual relations between genders. In the present paper have developed some methodological and technological processes of the method of disorientation of males *G. funebrana*.

To determine the speed of emanation of the components of the pheromone composition and longevity of the period of action of dispensators were applied formula: $\ln Y = \ln Y_0 - m\phi$ (for the component "Z8-12Ac") and $\ln X = \ln X_0 - k\phi$ (for the component "H-12Ac"). The results obtained are shown in Table 6.

Determining the speed of its release while the synthetic sexual pheromone out of dispensators by chromatographic method showed that after 30 days of exposure in their field remained only 3-5% of the initial pheromone injected uantity (Fig. 3).

Table 5. The biological effectiveness of the method of mass capture of males *Grapholitha funebrana*, depending on the longevity of application. (S = 5,4 ha)

Variants	I generation		II generation		Number of chemical treatments
	The number of males captured	Plums attacked (%)	The number of males captured	Plums attacked (%)	
Year 2008					
Standards	-	8,0	-	2,0	3
Experience	11696	2,6	6630	2,8	1
Year 2009					
Standards	-	6,0	-	2,0	2
Experience	9418	2,0	5100	2,4	1
Year 2010					
Standards	-	2,5	-	1,6	2
Experience	731	1,0	1748	2,0	0

Table 6. Diffusion speed of synthetic sexual pheromone components of the species *Grapholitha funebrana* out of the dispensator under field conditions

Variants	Component „Z8-12Ac"			Component „H-12Ac"		
	$\ln Y = \ln Y_0 - m\tau$			$\ln X = \ln X_0 - k\tau$		
	$\ln Y_0$	m	/ r /	$\ln X_0$	k	/ r /
Generation I	0,78	$9,6 \times 10^{-2}$	0,90	0,27	$8,6 \times 10^{-2}$	0,99
Generation II	1,04	$1,1 \times 10^{-1}$	0,99	0,34	$9,4 \times 10^{-1}$	0,99

Legend: Y, X – concentration of components (mg/cm^2);
τ – the length of time of exposure of dispensator;
/r/ – correlation coefficient.

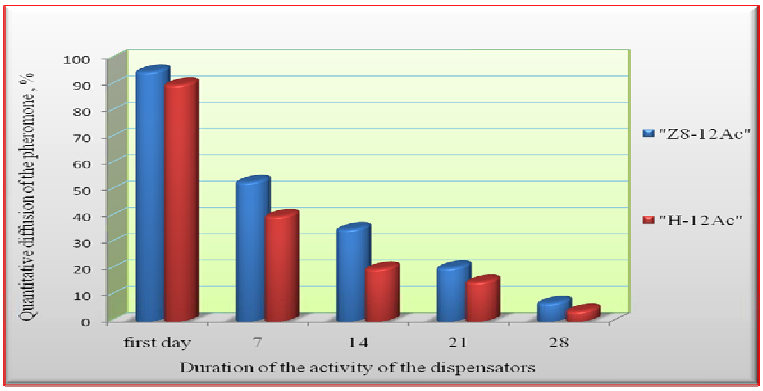


Fig. 3. Diffusion dynamics of the pheromone components *Grapholitha funebrana* species "Z8-12Ac" and "H-12Ac" in dispensator

Based on the results we conclude that dispensators developed can be applied under field conditions to test the method of disorientation of the males of the species *G. funebrana*.

Further testing was performed the disorientation method of males *G. funebrana* on the background of different population densities. 5 different versions have been exposed to testing. In each variant were applied every 500 pheromone dispensators displayed on plum tree branches at a height of about 2.0 m from the ground (for one preparative form to the tree). In the variants exposed to testing there was applied for 25.0, 20.0, 15.0, 10.0 and 5.0 g of synthetic sexual pheromone per hectare. Biological efficacy was assessed for each variant by setting indices of repression of male response to pheromone traps and reducing the rate of fruits attacked by weevils. The analysis of results showed that under the influence of the environment, saturated with synthetic sexual pheromone was essentially suppressed (97.3 to 99.6%) male's response to pheromone traps in all cases, without some generational difference.

The results of analysis of fruits also showed that there was a significant reduction of the damage caused. Thus, in all variants the rate of the attacked plums was within 2.0% - which did not exceed the economic threshold of damage. At the same time it was established, that on the witness sector the rate of plums was within the 13.0 to 15.6% (Table 7).

The analysis of the speed of sexual pheromone emanation out of dispensators was calculated the amount that were emanated daily on the experimental sectors. Thus it was demonstrated that following the application dose of 25.0 g/ha daily were emanated about 790.0 mg/ha. By applying the dose of 5.0 g/ha - diurnal releasing of pheromone out of dispensators was about 150.0 mg/ha. Based on the results we can conclude that after applying a 5.0 g/ha is possible to obtain biological efficacy equal to that obtained in the case of 25.0 g/ha.

Testing the method of disorientation of *G. funebrana* males under production took place in a plum orchard with the surface of 5.4 ha, belonging to "AGROBRIO" LLC, Bacioi village. There was applied the composition "[Z8-12Ac97% + E8-12Ac3%] (10%) + H-12Ac (90%)" (10 mg/dispensator). They were shown for 500 dispensators/ ha on the basis of 5.0 g/ha of pheromone during the development of a generation.

As a result was diminished essentially the density of the pest population *G. funebrana*. Thus, the essential suppression was obtained (97.3 to 99.2%) of male response to pheromone traps and a significant reduction in the rate of plums attacked (up to 1.0 to 2.8%). In witness, the rate of plums attacked constituted 13.0 to 15.6% (Fig. 4).

Table 7. The biological efficacy of the method of disorientation of the male *Grapholitha funebrana*, depending on the dose of synthetic sexual pheromone applied
(Composition of the pheromone: „[Z8–12Ac 97% + E8–12Ac 3%] (10%) + H-12Ac (90%)")

Variant	Dose of the pheromone applied (g/ha)	I generation		II generation		Emanation rate of pheromone of dispensation (mg/ha/day)
		Suppression of male response to pheromone traps (%)	Rate of attacked plums (%)	Suppression of male response to pheromone traps (%)	Rate of attacked plums (%)	
Witness	-	-	13,0	-	15,6	-
I	25,0	99,1	0	99,6	1,0	790,0
II	20,0	99,1	0	99,0	1,0	630,0
III	15,0	99,1	0	99,0	1,0	480,0
IV	10,0	98,8	1,0	99,2	1,4	320,0
V	5,0	97,3	1,0	97,3	2,0	150,0

Fig. 4. Images of the plum orchard where was tested the method of disorientation of males *Grapholitha funebrana* („AGROBRIO"LLC, Bacioi village, 2013)

Due to technological elements and methodological procedures developed it was demonstrated that the method of disorientation has a high biological effectiveness of reducing population density and species *G. funebrana* and is equal with effectiveness of application of 2-3 treatments with insecticides. It should be noted that during the development of two generations of the pest *G. funebrana* was not conducted any treatment with insecticides in the experimental orchard. This had a positive effect on the accumulation in the proper orchard of species of beneficial insects.

CONCLUSIONS

It was developed a new scheme for the synthesis of sexual pheromone of *Grapholitha funebrana* species;

There have been proposed a new synthetic sexual pheromone composition of species *Grapholitha funebrana* „**[**Z8–12Ac 97% + E8–12Ac 3%**]** (10%) + H-12Ac (90%)".

There have been developed a new pheromone dispensator for applying the method of disorientation of *Grapholitha funebrana* males.

It has been shown, under agricultural climate of the Republic of Moldova, phytophagous species *Grapholitha funebrana* develops two generations (of the third decade of April - until the first decade of September).

It was demonstrated that the application of mass capture of males *Grapholitha funebrana* in one and the same orchard during many years reduce significantly the pest population density and allows to protect the plum fruit without chemical treatments;

It was found that the method of disorientation of males *Grapholitha funebrana (*pheromone – "[Z8-12Ac97% + E8-12Ac 3%] (10%) + H-12Ac (90%)" - 5.0 g/ha/generation) allows to reduce the damage caused to plums with about 97.2%.

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ABSTRACT

With the development of a new scheme for the synthesis of sexual pheromone of *Grapholitha funebrana* pest, developing a new pheromone composition - "[Z8-12Ac97%+E8-12Ac 3%] (10%) + H-12Ac (90%)" and a new dispensator was possible to develop methodological and technological processes for applying their methods of capture mass and disorientation of males. It was shown that application of appropriate methods allow to substantially reducing the population density of the given pest, the significant reduction of the damage caused and obtainment of the new production of green plums.

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