RESEARCH ON THE POSSIBILITY OF USING SEMIOCHEMICAL PRODUCTS IN MONITORING THE SPECIES *MELIGETHES* (*BRASSICOGETHES*) *AENEUS* F.

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Abstract. In the autumn rapeseed crop, one of the great damage is caused by the rape beetle, *Meligethes* (*Brassicogethes*) *aeneus* F. The widespread use of the chemical method of control has led to the emergence of resistance to some insects. This paper presents the results obtained regarding the possibility of using semi-chemical products in monitoring this species and possibly in control it. During the three experimental years, the attractiveness of three substances was verified: allyl isothiocyanate, nonane and 2-phenyl-ethyl isothiocyanate. With these substances were formulated three types of dispensers that were placed on two types of traps: sticky yellow panels and white Delta type traps. All variants with dispensers made a higher number of catches compared to the control, the differences being statistically assured. In the period 2016-2018, out of the total catches, the control variant achieved 11.3%; dispenser 1 (allyl isothiocyanate + nonane) achieved 23.6%; dispenser 2 (2-phenyl-ethyl isothiocyanate + nonan) made 27.6%, and dispenser 3 (allyl isothiocyanate + 2-phenyl-ethyl isothiocyanate + nonan) made 37.5% of the total catches. The allyl isothiocyanate and 2-phenyl-ethyl isothiocyanate components used together have a synergistic effect so that between the catches made by dispenser 3 and dispenser 1 and dispenser 2, the differences are statistically assured.

Keywords: *Meligethes* (*Brassicogethes*) *aeneus F.,* monitoring, semi-chemical, yellow panels, Delta traps.

INTRODUCTION

The rape beetle, *Meligethes aeneus* F. (synonym *Brassicogethes aeneus* Fabricius) is widespread in the steppe and forest-steppe areas as well as in the regions of deciduous forests (beech, oak), areas with richer rainfall (Rîșnoveanu *et al*., 2012; Ursache *et al*., 2017). The species is polyphagous but prefers crucifers (Skellern and Cook, 2018). It is one of the most dangerous pests of rapeseed (Čuljak *et al*., 2016). At the European level, populations of this genetically differentiated species have been reported in many countries (Kazachkova and Meijer, 2007; Juhel *et al*., 2019

The species has only one generation per year and winters as an adult in the surface layer of the soil, at a depth of 2-3 cm, or under the plant debris left in the field and even in the litter of forests. In the spring to the end of April, the beetles leave the wintering places and fix themselves on the inflorescences of different plants from the spontaneous flora (*Ranunculus*, *Taraxacum* etc.) (Tălmaciu *et al*., 2009). After a short period, they pass on rapeseed or even on other cruciferous (cauliflower, cabbage, etc.). Adults attack the flower buds, feeding on pollen and nectar, internal organs of the flower, causing appreciable damage by gnawing the pistil, the flowers are sterile and fall prematurely. The critical attack period is the green-yellow phenophase when the pest causes the most damage.

After a period of feeding, mating and laying of eggs take place. Egg laying is staggered, starting with the first decade of May, until June. The eggs are laid in small perforations in the flower buds of rapeseed, mustard and various other crucifers. The larvae appear during the opening of the flower buds of crucifers, and their evolution lasts 3-4 weeks, and they feed on the flower organs. The larvae have two-stage of development. The newborn larva usually develops in closed flower buds, while the second-age larva feeds on open flowers and moves frequently among younger flowers (Veromann *et al*., 2014; Corda et al., 2018). When mature, the larvae pierce the wall of the flower buds and retreat into the soil for soaking in nymph lodges. After 10-12 days, adults appear at the end of June. After a short period of feeding on pollen from various plants, when the temperature is about 15°C, the adults begin to retreat into the soil for hibernation.

The best-known method of controlling this species is the chemical method. But, unfortunately, there have been some exaggerations, which has led to the fact that over time it has been found that the technique has a multitude of side effects. In the protection of rapeseed crops in recent times have been used especially insecticides from the group of synthetic pyrethroids and the group of neonicotinoids (Brandes et al., 2018). The use of this insecticides led to the emergence of the phenomenon of resistance. Resistance was first reported in 1999 in France (Hansen, 2003), then throughout Europe (Heimbach *et al*., 2006; Węgorek *et al*., 2009; Nauen *et al*., 2012; Zimmer *et al*., 2014; Riggi *et al*., 2016; Kaiser *et al*., 2017; Skellern and Cook, 2018; Stará and Kocourek, 2018).

Given the phenophases in which treatments are applied in rapeseed culture (flowering or at the end of flowering), insecticides affect useful entomofauna, pollinators and zoophagous (Veromann *et al*., 2009; Ulber *et al*., 2010).

Various parasites for *Meligethes aeneus* F. are listed in the literature (Büchi, 2002; Kraus and Kromp, 2002; Jonsson *et al*., 2005). For this species, the most common parasitoids on the European continent are three Hymenoptera from the *Ichneumonidae* family, these being: *Tersilochus heterocerus* Thomson, *Phradis interstitialis* Thomson and *Phradis morionellus* Holmgren (Kaasik *et al*., 2014; Berger *et al*., 2015; Šenglř, 2018). Another Hymenoptera has also been reported:

- *Ceraphronidae* (*Ceraphron bispinosus* Ness., *Ceraphron serraticornis* Kieff., *Ceraphron insularis* Kieff., and *Aphanogmus abdominalis* Thomson).
- *Platygastridae* (*Platygaster subuliformis* Kieff.).
- *Eulophidae* (*Omphale clypealis* Thomson)
- *Braconidae* (*Diospilus capito* Ness.) (Hokkanen, 2008; Uber *et al*., 2010; Šafář and Seidenglanz, 2018).

MATERIAL AND METHODS

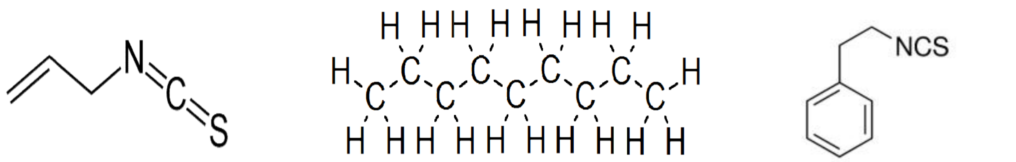
Meligethes (*Brassicogethes*) *aeneus* F. locates its food host plants, which include rapeseed, using a variety of chemical and visual signs of the plants.

Plants have complex biosynthetic pathways that lead to the release of volatile organic compounds, some of which are specific, and insects have evolved to use this volatiles as semiochemical clues in the food-plant location (Smart and Blight, 2000; Dicke and Baldwin, 2010). Plants volatiles are a combination of volatiles emitted by all green plants, including green-leafed volatiles, terpenes, and aromatic floral compounds. Still, some plant species also release genus-specific compounds, such as isothiocyanates released by Brassicaceae (Bruce *et al*., 2005). *Brassicogethes aeneus* is attracted to general floral compounds such as phenylacetaldehyde and indole with a possible phagostimulant role for insects (Smart and Blight, 2000). The chemical basis for this attraction has been tested in the laboratory using electrophysiological studies, and the most active compounds include 2-phenylethyl-, 3-butenyl- and 4-pentenyl-isothiocyanate, phenylacetaldehyde and indole (Cook *et al*., 2007b). Integrated pest control tools are currently being developed, which use semiochemicals to manipulate the behaviour of harmful insects and their natural enemies to ensure effective pest control in culture (Cook *et al*., 2007a; Mauchline *et al*. ., 2018).

The "Raluca Ripan" Institute of Chemistry at UBB Cluj-Napoca has a laborious research activity regarding the synthesis of numerous semiochemical products which, then, are tested in production conditions to establish their effectiveness in monitoring and control specific species of pests in agricultural ecosystems.

In the period 2016-2018, we tested the functionality of such semiochemical formulations to be used for monitoring the species *Meligethes* (*Brassicogethes*) *aeneus* F. The experimental lots were located in Pomârla, Botoşani County.

Three semiochemical formulations were tested for this experiment: allyl isothiocyanate, nonane and 2-phenyl-ethyl isothiocyanate (Fig. 1.).



izotiocianat de alil nonan 2-fenil-etil izotiocianatului

Fig. 1. Active compounds of attractant lures

Allyl isothiocyanate (synonym 3-isothiocyanatoprop-1-ene) is a volatile organic compound found in mustard, horseradish and other crucifers, which is readily soluble in water but very soluble in most organic solvents. It has also been tested for monitoring rapeseed fleas (Burgess and Wiens, 1980; Pivnick *et al*., 1992). The chemical formula of the product is: C_4H_5NS (structural formula: $CH_2 = CHCH_2N = C = S$).

Nonane is a higher alkane with the chemical formula C_9H_{20} and the restricted structural formula CH_3 (CH_2) $7CH_3$.

2-phenyl-ethyl isothiocyanate is an isothiocyanate having a phenethyl group attached to nitrogen, with the chemical formula C_9H_9NS and the restricted structural formula $C_6H_5CH_2CH_2NCS$. It is a compound that is found naturally in some crucifers.

Three types of dispensers were made with these components.

- Type 1 pheromone dispenser contains allyl isothiocyanate (94%) and nonane (99%). The evaporation rate of allyl isothiocyanate is 30 mg/day.
- Type 2 pheromone dispenser contains 2-phenyl-ethyl isothiocyanate (99%) and nonane (99%). The evaporation rate of 2-phenyl-ethyl isothiocyanate is 5 mg/day.
- Pheromone dispenser type 3 contains allyl isothiocyanate (94%), 2-phenyl-ethyl isothiocyanate (99%) and nonane (99%). The evaporation rate of allyl isothiocyanate is 30 mg/day, and 2-phenyl-ethyl isothiocyanate is 5 mg/day.

Pheromone dispensers consist of rectangular pieces of saturated felt with the attractive pheromone mixture inserted into polyethylene bags that protect the chemical compound from the weather and gradually release the pheromone. A sufficient amount was dosed on each felt for three weeks (21 days).

In the experimental part, two types of traps were used on which the dispensers were placed: glued yellow panels and Delta traps.

Each year there were the following experimental variants that were placed in three repetitions:

- Control glued yellow panels and Delta traps.
- Dispenser 1 on glued yellow panels and Delta traps
- Dispenser 2 on glued yellow panels and Delta traps
- Dispenser 3 on glued yellow panels and Delta traps

The experiment was set up in the first days of May, in the plot cultivated with the hybrid ES Alonso (the earliest hybrid from the experimental plots), the period in which the rape beetle was in full flight activity.

The catches were read twice a week for three weeks, mark down the catches made.

When interpreting the results, the number of catches made by each dispenser was analysed depending on the type of panel (yellow panel or Delta trap) but also cumulated on both types of panels used.

The results obtained were statistically processed using the Duncan test.

RESULTS AND DISCUSSION

Table 1 shows the catches made in each variant depending on the type of trap used, and the total catches made both on the yellow panels and in the Delta traps.

Table 1.

The total number of captures made

Variant	Yellow panels			White trap Delta			Total		
	2016	2017	2018	2016	2017	2018	2016	2017	2018
Martor / Control	145	133	204	41	35	71	186	168	275
Dispenser 1	255	248	318	154	141	201	409	389	519
Dispenser 2	253	336	334	202	169	250	455	505	584
Dispenser 3	337	464	491	269	220	314	606	684	805
Total	990	1181	1347	666	565	836	1656	1746	2183

In the control variant on the yellow panels, the number of catches was between 133 specimens, in 2017, and 204 samples in 2018. The highest number of catches made by a panel over the entire experimental period was 82, and the fewest catches there were 21. In the white Delta type traps, the number of catches was much lower, ranging between 35 (in 2017) and 71 catches (in 2018). The number of catches/traps was between 8 and 31 specimens. From the presented data, it can be seen that the yellow panels have a much higher power of attraction of the adults of the shiny beetle of rapeseed compared to the white traps type Delta. The number of catches made by the yellow panels was 3.5 times higher in 2016, 3.8 times in 2017 and 2.9 times in 2018, compared to Delta traps. The literature also shows that adults of *Meligethes aeneus* are attracted to the yellow colour of the petals. By placing yellow and white traps, it was found that a small number of catches are made on the white ones. Therefore studies are being made to obtain a rape whose petals are of a different colour by introducing the key gene from other species (Cook et al. , 2006; Döring et al., 2012; Cook et al., 2013).

In the version where dispenser 1 was placed compared to the control version, each year the number of catches was higher on both the yellow panels and the Delta traps, which indicates that the tested product (allyl isothiocyanate + nonan, with an evaporation rate 30 mg/day) can attract adults of *Meligethes* (*Brassicogethes*) *aeneus*. Compared to the control variant, the number of catches on the yellow panels was 1.8 times higher in 2016, 1.9 times higher in 2017 and 1.6 times higher in 2018. Cumulatively over the three years, the average increase was 1.7 times higher. On Delta traps, compared to the control, there was an increase in the number of adults caught 3.8 times in 2016, 4 times in 2017 and 2.8 times in 2018. Over the three experimental years, the average increase in catches on Delta traps of this variant was 3.4 times higher. The captures made show that the tested formulation has the power of attraction for the adults of the monitored species.

On the yellow panels the number of catches was between 248 in 2017 and 318 in 2018, and on the Delta traps between 141 in 2017 and 201 in 2018. During the three years, the number of catches on a yellow panel was between 77 and 113 specimens, and between 37 samples and 82 adults were caught on Delta traps.

With dispenser 2 in which the active component is 2-phenyl-ethyl isothiocyanate + nonane, with an evaporation rate of 5 mg/day, the number of catches on the yellow panels was between 253 in 2016 and 336 in 2017, and on the Delta traps between 169 in 2017 and 250 in 2018. During the three years, the number of catches on a yellow panel was between 67 specimens and 133, and between Delta trapped between 39 specimens 97 adults were caught.

Also in this variant, every year the number of catches was higher both on the yellow panels and on the Delta traps compared to the control. Compared to the control variant, the number of catches on the yellow panels was 1.7 times higher in 2016, 2.5 times in 2017 and 1.6 times in 2018, and the average increase over the three years was 1.9 times higher. On Delta traps, the rise in the number of catches is 4.9 times in 2016, 4.7 times in 2017 and 3.5 times in 2018, and the average growth over the three years was 4.2 times higher. The data obtained demonstrate the efficacy of the tested product.

Compared to dispenser 1, on the yellow panels the catches made in 2016 and 2018 are substantially equal (2 fewer catches in 2016 and 16 more catches in 2018), instead, in 2017 in this variant were captured with 88 more individuals. Much more adults were caught on Delta traps in this variant compared to dispenser 1, 48 more in 2016, 28 individuals in 2017 and 49 specimens in 2018.

From the data presented in table 1 it can be seen that the two components of dispenser 3 (allyl isothiocyanate 2-phenyl-ethyl isothiocyanate nonan) have a synergistic effect, the catches on both types of traps are numerically superior to those made in previous versions.

On the yellow panels the number of catches was between 337 in 2016 and 491 in 2018, and on the Delta traps between 220 in 2017 and 314 in 2018. During the three years, the number of catches on a yellow panel was between 101 specimens and 204, and between 57 specimens and 122 adults were caught on Delta traps.

Compared to the control variant, the number of catches on the yellow panels was 2.3 times higher in 2016, 3.5 times in 2017 and 2.4 times in 2018 (the multiannual average being 2.7 times higher), and on Delta traps 6.6 times higher in 2016, 6.3 times in 2017 and 4.4 times in 2018 and the average growth over the three years was 5.5 times higher.

Compared to dispenser 1, the number of catches on the yellow panels was 1.3 times higher in 2016, 1.9 times in 2017 and 1.5 times in 2018 (the average for the three years is 1.6 times higher), and on Delta traps 1.7 times higher in 2016, 1.6 times in 2017 and 2018.

Compared to dispenser 2, the number of catches on the yellow panels was 1.3 times higher in 2016, 1.4 times in 2017 and 1.5 times in 2018, and on the Delta traps about 1.3 times higher each year.

If we analyse the total number of catches made cumulatively on the two types of traps during the study period in the four experimental variants, we see an increase from one year to another. This shows that the dynamics of the numerical density of this species has an ascending allure.

In 2016, 1656 specimens were captured from the four variants, of which 990 on the yellow panels and 666 on the Delta traps. In 2017 there were 1746 catches, of which 1181 on the yellow panels and 565 on the Delta traps, and in 2018 there were 2183 catches, of which 1347 on the yellow panels and 836 on the Delta traps.

Figure 2 shows the total number of catches made in the three years for each experimental variant. Experimentally, 629 specimens were noted on control variant (of which 482 on yellow panels and 147 on Delta traps), 1317 adults at dispenser 1 (of which 821 on yellow panels and 496 on Delta traps), a total of 1544 at dispenser 2 (of which 923 on yellow panels and 621 on Delta traps) and 2095 specimens at dispenser 3 (of which 1292 on yellow panels and 803 on Delta traps).

In the three experimental years, 5585 adults of *Meligethes* (*Brassicogethes*) *aeneus* F. were caught, of which 3518 on yellow traps (representing 63% of total catches) and 2067 on Delta traps (representing 37% of total catches).

If we express as a percentage the number of catches made in each experimental variant over the entire monitoring period, the situation is as follows:

In the control version, the catches made on the yellow panels represented 13.7%, and on the Delta traps 7.1%, and on the total experiment, they represented 11.3%.

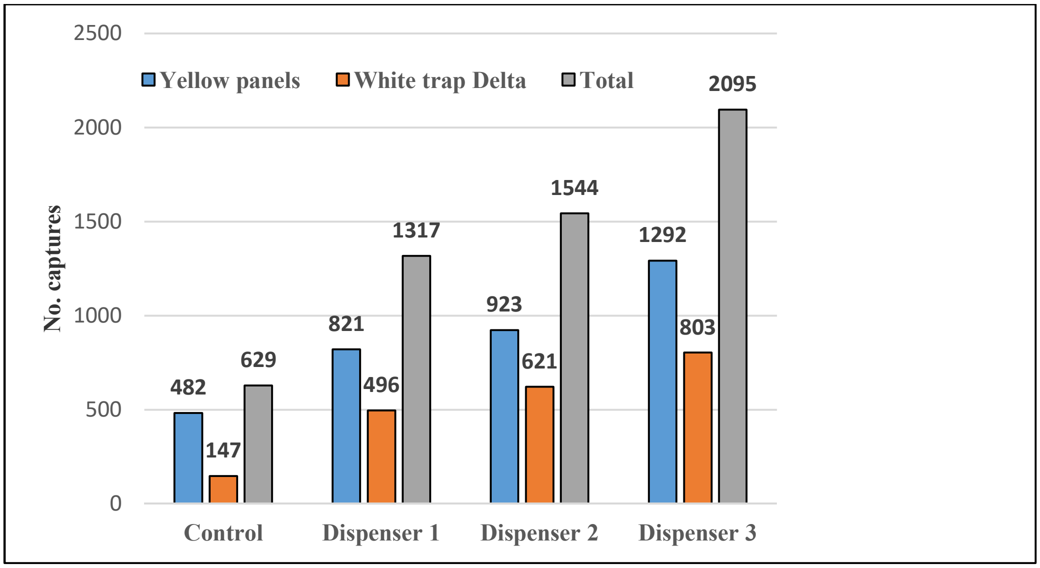


Fig. 2. Total catches/variant made during 2016-2018

On dispenser 1, the catches made on the yellow panels represented 23.3%, and on the Delta traps 23.9%, and on the total experiment, they represented 23.6%.

On dispenser 2, the catches made on the yellow panels represented 26.3%, and on the Delta traps 30.2%, and on the total experiment, they represented 27.6%.

On dispenser 3, the catches made on the yellow panels represented 36.7%, and on the Delta traps 38.8%, and on the total experiment, they represented 37.5%.

Between the percentage of catches of dispenser 1 and 2, the difference is only four percentage points, instead, dispenser 3 is detached by the catches made.

Figure 3 is a graphical representation in terms of a comparison between the percentage of total catches made by the tested variants cumulated on both types of traps used during 2016-2018 (we did not take into account the catches made in the control version).

With the three pheromonal formulations in 2016, a total of 1470 catches were made on the two types as traps (845 on yellow panels, 625 on Delta traps). Of the total catches, 27.9% were with dispenser 1 (409 adults), 30.9% with dispenser 2 (455 adults) and 41.2% with dispenser 3 (606 adults).

With the yellow and Delta traps in 2017, 1578 catches were made (1048 on yellow panels, 530 on Delta traps), of which 24.7% with dispenser 1 (389 adults), 32% with dispenser 2 (505 adults) and 43.3% with dispenser 3 (684 adults).

In 2018, 1098 catches were made in the variants with the tested pheromone products (1143 on yellow panels, 765 on Delta traps). Of the total catches, 27.2% were with dispenser 1 (519 adults), 30.6% with dispenser 2 (584 adults) and 42.2% with dispenser 3 (805 adults).

During the three years, 4956 catches were made (3036 on yellow panels, 1920 on Delta traps). Of the total catches, 26.5% were made with dispenser 1 (1317 adults), 31.2% with dispenser 2 (1544 adults) and 42.3% with dispenser 3 (2095 adults).

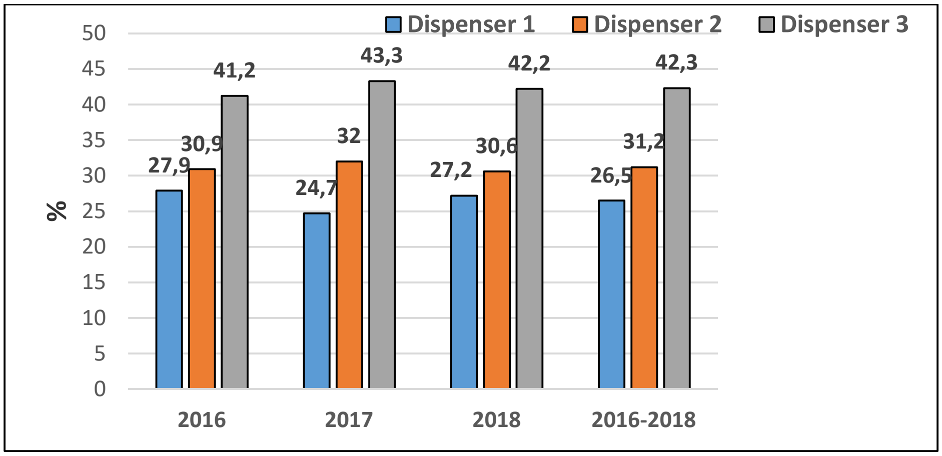


Fig. 3. Total catches made by the three dispensers during 2016-2018 (%)

Table 2 shows the results of the statistical interpretation of the average number of catches made during the three experimental years by a trap depending on the experimental variant.

All variants with dispensers had differences from the control, which were statistically assured. The average number of catches made by dispenser 1 is higher than the control, the difference being distinctly significant positive. Dispenser 2 and 3 made more captures compared to the control, the difference being very significant positive.

The Duncan test shows that the differences between the control and the dispenser variants are statistically assured. The differences between dispenser 1 and dispenser 2 are not statistically confirmed. Instead, the differences between dispenser 3 and dispenser 1, as well as between dispenser 3 and dispenser 2, are statistically assured.

Table 2.

Influence of treatment on the number of catches of rape beetle (2016-2018)

Dispenser	Average/trap	Difference to control	The significance of difference	Duncan Test
Control	69.89	0.00	Mt.	A
Dispenser 1	146.33	76.44	**	B
Dispenser 2	171.56	101.67	***	B
Dispenser 3	232.78	162.89	***	C
DL (LSD) (p 5%)			33.47	
DL (p 1%)			50.68	
DL (p 0.1%)			81.42	

CONCLUSIONS

In Pomârla, Botoşani county, the species *Meligethes* (*Brassicogethes*) *aeneus* F. is a frequently encountered pest, with a tendency to increase the numerical density. The total catch made during the monitoring period was 1656 specimens in 2016, 1746 catches in 2017 and 2183 catches in 2018.

The simple yellow panels that are used by farmers in monitoring the species *Meligethes* (*Brassicogethes*) *aeneus* F. in the period 2016-2018 made a total of 482 catches, representing 13.7% of the total catches made in the experimental plot. During three weeks of testing, the catches made on a panel ranged from 21 to 82 specimens. Delta white traps are not recommended to be used, the catches made by them are meagre, representing only 7.1%.

By using semi-chemical products synthesised at the "Raluca Ripan" Institute of Chemistry at UBB Cluj-Napoca, during the experimental period, an increase in catches was achieved regardless of the type of trap on which the dispenser was placed. All variants with dispensers had differences from the control, which were statistically assured.

Dispenser 1 (allyl isothiocyanate + nonan) made 23.6% of the total catches. Compared to the control variant, it achieved an increase of catches over the three experimental years 1.7 times higher on the yellow panels and 3.4 times higher on the white Delta type traps, the difference being distinctly significant positive.

Dispenser 2 (2-phenyl-ethyl isothiocyanate + nonan) made 27.6% of the total catches. Compared to the control variant, it made 1.9 times more catches on the yellow panels and 4.2 times more on the Delta traps, the difference being very significantly positive.

At dispenser 3 (allyl isothiocyanate + 2-phenyl-ethyl isothiocyanate + nonan) 37.5% of the total catches were made. Compared to the control variant, the increase in catches was 2.7 times more catches on the yellow panels and 5.5 times more on the Delta traps, the difference being very significantly positive. The components have a synergistic effect; the catches on both types of traps are numerically superior to those made in the previous pheromone variants. The differences between dispenser 3 and dispenser 1, as well as between dispenser 3 and dispenser 2 are statistically assured.

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