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THE TESTING OF SOME PRODUCTS IN ORDER TO MONITOR THE *Cameraria ohridella* DESCHKA-DIMIĆ SPECIES (*Lepidoptera*: *Gracilariidae*)

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Abstract

The horse-chestnut leaf miner - Cameraria ohridella Deschka-Dimić (Order: Lepidoptera, Family: Gracilariidae) is a serious threat to chestnut trees in cities, as well as a potential threat to biodiversity on the whole. In the period 2016-2017, a research was carried out to monitor and test certain products against the Cameraria ohridella species. These tests were carried out in two locations in Cluj-Napoca and Caracal. Pheromone baits traps were used in three variants (V1, V2, and V3) and were installed in four different places in U.A.S.V.M. Cluj-Napoca Campus and three other places in Constantin Poroineanu Park in Caracal. The synthesis of the pheromone baits was done at Babes-Bolyai University, Cluj-Napoca. During the study it has been noted that the horse-chestnut leaf miner, generally develops three generations/year. In the Caracal area, the maximum population density is reached at the end of July - the first decade of August, and in Cluj-Napoca area in the second decade of August. The total number of adults captured during the period 2016-2017, in Cluj-Napoca is 95756 and 15977 in Caracal.

Key words*: pest, horse-chestnut leaf miner, traps, horse-chestnut.*

INTRODUCTION

Horse-chestnut (*Aesculus hippocastanum* L.) is a large, decorative tree, commonly grown in urban green spaces, whose foliar apparatus is infested with a new pest, the horse-chestnut leaf miner - *Cameraria ohridella* Deschka-Dimić (Order: *Lepidoptera*, Family: *Gracilariidae*). It is a serious threat to chestnut trees in cities, as well as a potential threat to biodiversity on the whole (Paterska et al., 2017). This species was first reported in 1985 in Macedonia (Simova-Tosic and Filov, 1985) and described and classified by Deschka and Dimić (1986).

The favorable climatic conditions, ease of adaptation, low number of specific parasites, *Penobius saulius* Walter, *Closterocerus trifasciatus* Westword, *Minotetrastichus frontalis* Ness (Pocock and Evans, 2014), *Minotetractichus frontalis, Pnigalio agraules* are responsible for a 37% parasitism (Bhatti and Shaw, 2013), and the most important ones are on the hibernating pupae (Nováková et al., 2016; Kopacka and Zemek, 2017). Perju et al. (2004, 2007) and Oltean et al. (2006, 2015) identified 16 species of *Hymenoptera* parasitoid in Cluj area. From the family *Pteromalidae* was identified: *Pteromalus semotus* Walk. Most of them belong to the family *Eulophidae*: *Minotetrastichus frontalis* Ness., *Pnigalio agraules* Walk., *Pnigalio pectinicornis* L., *Closterocerus trifasciatus* Westw., *Sympiesis sericeicornis* Ness., *Pediobius saulius* Walk., *Minotetrastichus platanellus* Merc., *Baryscapus nigroviolaceus* Ness., *Chrysocharis* sp., *Closterocerus lyonetiae* Ferr., *Pediobius* sp., *Pnigalio soemius* Walk., *Pnigalio* sp. Was reported also a specie of the family *Encirtidae*, but

unidentified. The parasited larvae percentage ranged from 5.7-8.9%, and on pupae had values between 6.1-10.4%. The percentage of parasited pupae is much higher in the second period of biological cycle, especially in pupae of the second generation. These are the conditions that offer the pest the possibility of rapidly multiplying and spreading, covering almost the whole of Europe: Serbia (Dimić and Mihajlović, 1993), Germany (Butin and Füfrer, 1994; Heitland and Freise, 2002), Bulgaria (Draganova and Tomov, 2000; Trenchef et al., 2000), Sweden (Gilbert et al., 2005), Lithuania (Ozolinćius, 2010), Belgium (Prince and Prince, 2001).

In our country this pest was reported in the Western area - Lovrin/Timişoara, in 1996 by Şandru (Şandru, 1998; Vişoiu and Poşta, 2000). Then, this pest spread to the central area (Perju, 2000; Perju and Oltean, 2001; Perju et al., 2003), southern area of the country (Perju et al., 2002; Bădescu, 2003) and in the eastern part of the country (Perju et al., 2003). Lately, the insect has expanded its habitat and it was reported in Bucharest, Constanţa, Iaşi (Perju, 2002, 2003; Vişoiu and Poşta, 2000; Bădescu, 2003; Oltean et al., 2006).

In order to prevent and mitigate the effects of this pest it is necessary to follow the biological cycle (Talmaciu et al., 2017), to detect trends in population dynamics, to monitor (Oltean et al., 2005; Niculescu and Mitrea, 2016), and to take the appropriate measures by different methods. Bioproducts based on *Beauveria bassiana* and *Metarhizium anisopliae* are recommended for bio-control of this species (Schemmer, 2016; Barta, 2018) in order to protect useful entomofauna (Kopacka and Zemek, 2017; Mösch et al., 2018). The chemical control is difficult, costly, with environmental effects (Pop and Oltean, 2007; Pop et al., 2007, 2008; Tsygankova et al., 2015), for example, fipronil and neonicotinoids affect the useful entomofauna, especially bees (Pisa et al., 2015). For this reason, three types of pheromone baits were synthesized and tested in terms of their functionality in catching males.

Considering the possibility of using sex attractant pheromones in order to monitor the population numerical density dynamics, to notify of the chemical treatments for control and especially to control by the mass catching of males, we considered that the synthesis and testing of the attractiveness of the three pheromone baits variants is of great relevance, and they can be used in the integrated control programs. The synthesis of pheromone baits was performed at Babes-Bolyai University (B.B.U.) Cluj-Napoca, and the testing of their functionality was carried out in two locations: Cluj-Napoca and Caracal.

MATERIALS AND METHODS

The composition of the pheromone baits for *Cameraria ohridella* Deschka-Dimić is as follows:

- Fer1= E8, Z10-14 Ald (1 mg/bait);
- Fer2= E8, Z10-14 Ald (0.5 mg/bait);
- Fer3= E8, Z10-14 Ald + E10 – 12 Acetate (0.5 + 0.5 mg/bait) (E10 - 12 Acetate from V3 shows synergism in *Phyllonorycter blancardella*).

Traps with pheromone baits (Fer1, Fer2, and Fer3) were installed in different locations. In Caracal, the traps were located in three places, in three reoccurrences: the Stadium Area, the Lake Area, and the Main Alley in Constantin Poroineanu Park in Caracal.

In Cluj-Napoca, the traps were located in the University of Agricultural Sciences and Veterinary Medicine (U.A.S.V.M.) Cluj-Napoca Campus, in four places (in four reoccurrences in 2016 and in three reoccurrences in 2017): the Back of the Assembly Hall, Pedology, Shop and Platform.

Figure 1. Trap installation in Constantin Poroineanu Park in Caracal (original)

The traps were located on 19.05.2016 and 10.05.2017 in the U.A.S.V.M. Cluj-Napoca Campus and on 19.06.2016 and 26.04.2017 in Constantin Poroineanu Park in Caracal (Figure 1). The pheromone bait capsule was changed to a 6 weeks interval. The catching traps were read weekly in Constantin Poroineanu Park in Caracal, but also in the U.A.S.V.M. Cluj-Napoca Campus (Figure 2).

Figure 2. *Cameraria ohridella* Deschka-Dimić catch detail in the U.A.S.V.M. Cluj-Napoca Campus (original)

RESULTS AND DISCUSSIONS

The results obtained as regards the monitoring of the *Cameraria ohridella* Deschka-Dimić adults flight and the functionality of the pheromone variants tested are shown in Figures 3-6.

From the data shown in Figures 1 and 2, it appears that in the U.A.S.V.M. Cluj-Napoca Campus, in the installed traps there were captured a total number of 58613 adults during the entire monitored period in 2016, respectively a total number of 37143 in 2017. In both years, the largest number of adults were captured in the first half of August, a period overlapping the second and third generation flight. In 2016 the flight of adults to the hibernate generation began at the beginning of the last decade of May. This year there were two highlights of the flight curve, the first peak being in the first decade of July, and the second in the last decade of August. At the peak of August, the catches made accounted for more than 40% of the total annual. In 2017, when temperatures were higher than the lower thermal threshold since the end of April, the first adults were reported in pheromone traps in the first decade of May. Regarding the flight curve, three maximums were recorded: the second decade of June, the first decade of July and the second decade of August. In both years the adult flight ended in the end of the first decade of September.

If we compare the total number of catches taken on the trapping locations, it is found that it the point next to the Pedology discipline, the largest number of catches was recorded each year: 18479 samples in 2016 (representing about 32% of the total catches) and 14762 samples in 2017 (representing about 40% of the total catches). Instead, the lowest number of catches was recorded on the campus platform: 11722 samples in 2016 (representing about 20% of the total catches) and 10682 samples in 2017 (representing about 21% of the total catches). This situation is explained by the fact that from the campus platform leaves are gathered every autumn and thus it is destroyed the hibernating biological reserve, while near Pedology the fallen leaves remain on the bed of the watercourse crossing the campus. This aspect shows the importance of gathering and destroying fallen leaves.

Figures 3-4 show the evolution of catches noted for the three pheromone formulations tested.

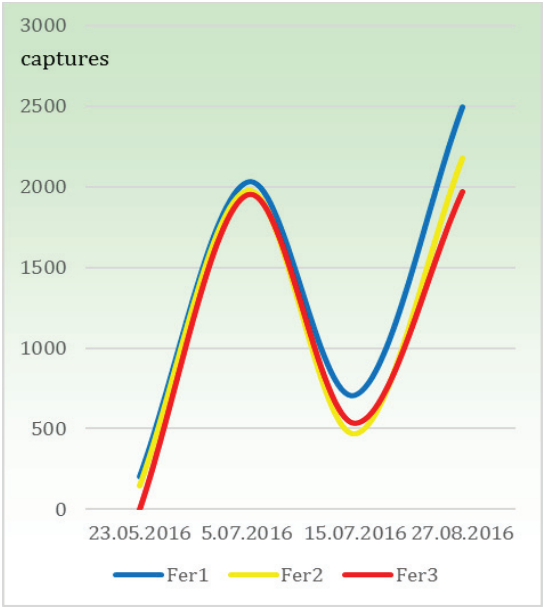

Figure 3. Evolution of catches (*Cameraria ohridella* Deschka-Dimić) in the U.A.S.V.M. Cluj-Napoca Campus, 2016

The data show that in 2016 the largest number of adults was captured in V1 pheromone traps, with a total of 21720 adults (representing about 37% of the total catches). In V2 pheromone traps, 19053 adults (representing about 33%) were captured, and 17840 adults (representing about 30% of the total catches) were captured in V3 pheromone traps

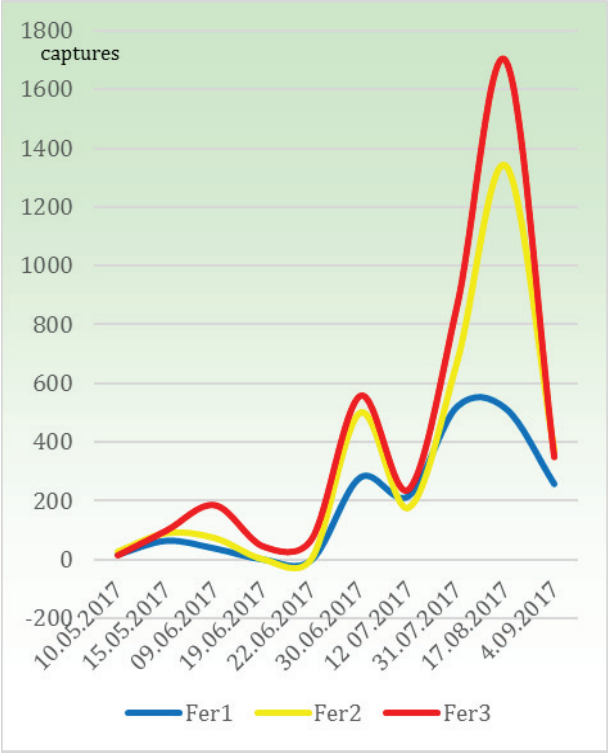

Figure 4. Evolution of catches (*Cameraria ohridella* Deschka-Dimić) in the U.A.S.V.M. Cluj-Napoca Campus, 2017

In 2017, the largest number of adults was captured in V3 pheromone traps, with a total of 16455 adults (representing about 44% of the catches), followed by V2 pheromone traps, with a total of 13034 adults (representing about 35%), and the smallest number of catches were in V1 pheromone traps, with a total of 7654 adults (representing about 21% of the total catches).

Differences between the pheromone variants tested during the two experimental years may be due to climatic differences between the years in terms of temperature, precipitation and light intensity, aspects also showed in other specialized works. Thus, Nowinszky and Puskás (2013) show that trap functionality depends on the dynamics of climatic factors, and Nowinszky et al. (2017) also shows the effect of light on trap functionality. By accumulating the catches made in the two years of research by the three pheromone formulations, we find that out of the 95756 adults captured, 34295 adults (36% of the total) were captured in V3, 32087 adults (34% of the total) were captured in V2, and 29374 adults (31% of the total) were captured in V1.

From the data shown in Figures 5 and 6 it results that in Constantin Poroineanu Park in Caracal, in the installed traps, a total number of 3009 adults were captured during the entire monitored period in 2016 and a number of 12977 adults in 2017. In this location, the numerical population density is lower than in the Cluj area. In 2016, the flight of adults of the hibernate generation began at the beginning of the first decade of May. Two flight curve peaks were reported, the first in the last decade of June, and the second in the first decade of August. In 2017, the first adults were reported in the first decade of May, a situation comparable to that reported in Cluj. Regarding to the flight curve, there were only two maximums: the second decade of June and the last decade of August. At the peak of August, the catches made accounted for over 45% of the total annual. In this area the maximum catches are made at the end of July - the beginning of August, compared to Cluj area, where this takes place about two weeks later. In 2016 adult flight ended in the last decade of September, and in 2017 towards the end of the first decade of September.

In 2016, the largest number of adults were captured in V3 pheromone traps, namely 1086 adults (representing about 36% of the total catches), then in V1 pheromone traps, with 1065 adults (representing about 35% of the total catches), and the smallest catches were in V2 pheromone traps, with a number of 858 adults (representing about 29% of the total catches).

In 2017, the largest number of adults were captured in V2 pheromone traps, namely 5282 adults (representing about 41% of the total catches), then in V3 pheromone traps with a total of 4026 adults (representing about 31% of the total catches), and the fewest catches were in V1 pheromone traps, which captured 3669 adults (representing about 28% of the total catches). We can notice that in this locality the attraction power of pheromone formulations

also showed differences from one year to the next, as was also noted in the Cluj area. This confirms the influence of climatic factors on the functionality of various pheromone formulations.

In this area out of the total of 15986 adults captured during the two years, 38% are in V2 variant (6140 adults), 32% are in V3 variant (5112 adults), and 30% of the catches were made in V1 variant (4734 adults).

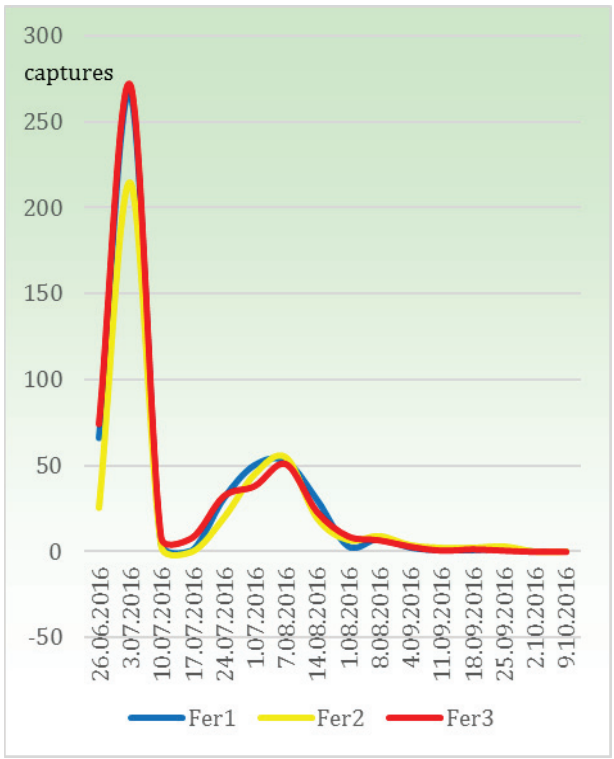

Figure 5. *Evolution* of catches (*Cameraria ohridella* Deschka-Dimić) in Constantin Poroineanu Park in Caracal, 2016

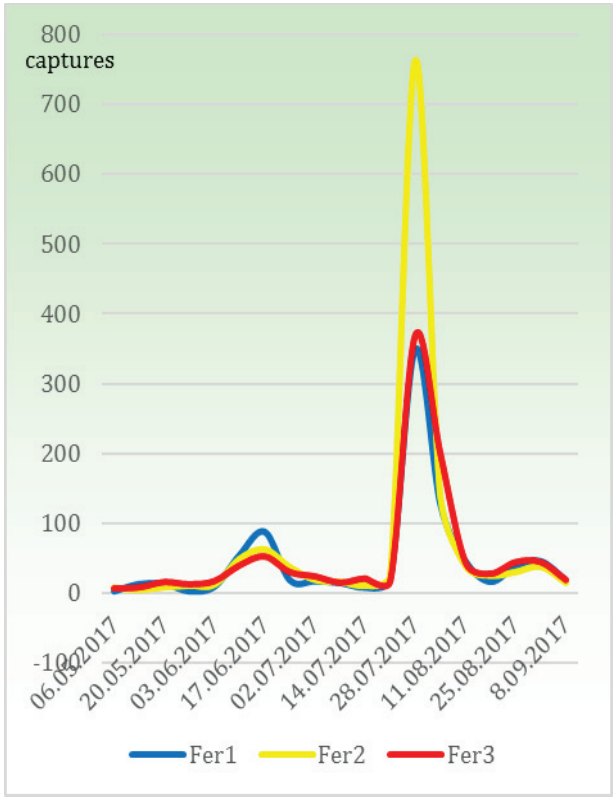

Figure 6. Evolution of catches (*Cameraria ohridella* Deschka-Dimić) in Constantin Poroineanu Park in Caracal, 2017

Tables 1 and 2 show the statistical interpretation based on the Duncan test of the results obtained with regard to the influence of the experimental area characteristics and the climatic conditions specific to each experimental year.

Table 1. The efficacy of pheromone variants in catching the *Cameraria ohridella* species, depending on location

Variant		Average number of catches	%	Difference	Significance	Duncan Test
Cluj-Napoca	**average**	4505.71	100.0	0.00	C	
	Fer1	3990.63	88.6	-515.08	o	B
	Fer2	4554.00	101.1	48.29	-	C
	Fer3	4972.50	110.4	466.79	*	C
Caracal	**average**	888.13	100.0	0.00	C	
	Fer1	789.00	88.8	-99.13	-	A
	Fer2	1023.38	115.2	135.25	-	A
	Fer3	852.00	95.9	-36.13	-	A

LSD (p 5%) - 449.06
LSD (p 1%) - 610.37
LSD (p 0.1%) - 817.46

DS - 450.10

Table 2. The influence of climatic conditions on the efficacy of pheromone variants in catching the *Cameraria ohridella* species

Variant		Average number of catches	%	Difference	Significance	Duncan Test
2016	average	2609.38	100.0	0.00	C	
	Fer1	2892.50	110.9	283.13	-	CD
	Fer2	2524.63	96.8	-84.75	-	BC
	Fer3	2411.00	92.4	-198.38	-	B
2017	average	2784.46	100.0	0.00	C	
	Fer1	1887.13	67.8	-897.33	ooo	A
	Fer2	3052.75	109.6	268.29	-	DE
	Fer3	3413.50	122.6	629.04	**	E

LSD (p 5%) - 449.06
LSD (p 1%) - 610.37 DS - 450.10
LSD (p 0.1%) - 817.46

It was found that significant differences between tested pheromone variants occur only in the case of a large number of capture. The best pheromone have been proven to be (Fer3) and (Fer2) forms, with significant differences from formulation (Fer1). In the case of the Caracal experience, there are no significant differences between pheromone variants, probably because of the lower number of catches. Comparing pheromone efficiency over the two experimental years, it can be noticed that although in 2016 Fer1 recorded the most catches, with no significant difference from the other two formulations, in 2017 it was the least effective. In 2017 the most potent pheromone was Fer3 (3413 catches).

CONCLUSIONS

The horse-chestnut leaf miner, *Cameraria ohridella* Deschka-Dimić, generally develops three generations/year.

In the Caracal area, the maximum population density is reached at the end of July - the first decade of August, and in the Cluj area in the second decade of August.

The total number of adults captured during the period 2016-2017, in the U.A.S.V.M. Cluj-Napoca Campus is 95756 and 15977 in Constantin Poroineanu Park in Caracal.

It is noted that in Caracal the numerical population density is lower compared to that in the Cluj area, this parameter being determined by the climatic zones and the strategies applied in the control of this pest.

In the U.A.S.V.M. Cluj-Napoca Campus, the largest number of adults captured during the two experimental years was in V3 pheromone traps (E8, Z10-14 Ald + E10 – 12 Acetate; 0.5+ 0.5 mg/bait), followed by V2 pheromone traps (E8, Z10-14 Ald; 0.5 mg/bait), and the lowest catches were made by V1 pheromone traps (E8, Z10-14 Ald; 1 mg/bait).

In Constantin Poroineanu Park, the largest number of adults captured were in V2 pheromone traps, followed by V3 pheromone traps, and the lowest number of catches was in V1 variant.

The functionality of the various pheromone components is dependent on the climatic characteristics specific to each year.

Pheromones could be used in monitoring, forecasting and notification of chemical treatments, as well as in direct action of combat by mass catching of adults.

The gathering and destruction of fallen leaves contribute to reducing the biological reserve of *Cameraria ohridella* Deschka-Dimić species.

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