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Summary of the PhD thesis

**MONITORING, MORPHOLOGY, BIOLOGY, ECOLOGY
AND COMBATING OF SPECIES *CAMERARIA*
OHRIDELLA DESCHKA-DIMIĆ FROM SOME URBAN
GREEN SPACES IN THE SOUTH-EAST OF OLTENIA**

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SUMMARY

Key words: *Cameraria ohridella* Deschka-Dimić, monitoring, morphology, biology, ecology, combating, ornamental chestnut

Green spaces represent a functional category within the localities, whose specificity is determined by the vegetation, which is the main component of the **recreational areas**.

The variation in climatic conditions during the last period has led to the distribution of the species *Cameraria ohridella* Deschka-Dimić in Europe and in our country. The chestnut leaf mining moth produces damage to the ornamental chestnut by decreasing the decorative value. The paper presents research activities performed in order to obtain new information concerning the current status, the distribution of pest in urban ecosystems, to find the most effective methods of control and biological combating that represent the future efficient solutions for invasion problems in safe conditions for humans and environment.

In combating the pest there are many difficulties that are due to the functions of urban green spaces.

The thesis is structured in 6 chapters and contains 62 tables, 82 photos and 98 figures, of which 68 photos and 96 figures are original.

Chapter I. "Current state of research regarding the CAMERARIA OHRIDELLA DESCHKA-DIMIĆ chestnut mining moth" presents data from the specialized literature from the perspective of global distribution, systematic classification, biology, morphology of species *Cameraria ohridella* Deschka-Dimić and *Cameraria aesculisella* (Riley, 1891), ecology, flight orientation, host plants and prevention and combating measures. The limits of this species' distribution area on the European continent are represented to the east by Russia (GNINENKO, 2004; IZHEVSII, S.S., 2008; KASHTANOVA, 2009) to the west by Spain (VILLALVA and DEL ESTAL, 2003), to the north by Finland (BUSZKO, 2006) and to the south by Turkey (CEBECI and ACER, 2007). The main areas of geographical distribution in our country are: Banat, Crişana, Oltenia, Moldova, Muntenia, Transylvania.

The adult has a body of 4-5 mm in length and the span of the front wings is 6-7 mm, and the background color is yellowish. The egg is lenticular, transparent, white, with a diameter of 0.2-0.3 mm, length 0.3-0.4 mm. The larva presents an elongation of the body, obviously, thinner in the posterior. The cephalic capsule is yellowish, triangular, chitinated,

flattened. The nymph is of the obtect type, maroon-brown in color and has a length of 4-5 mm; the legs are oriented backwards and attached to the body.

Chapter 2. "Particularities of the natural environment in which the experiment took place" contains the geographical location and the general characterization of the area in the south-east of Oltenia, Constantin Poroineanu Caracal Park where the research activities were performed.

Chapter 3. "Objectives of research" In order to reduce the population of species *Cameraria ohridella* that was studied, it is necessary to establish broad strategies for the prevention and combating of pest, which consist of the inclusion of alternative methods in order to reduce the disadvantages of chemical methods for humans.

- ✓ Climatological analysis of the area during the period 2016-2018
- ✓ Studying aspects of development stages from the point of view of the external morphology.
- ✓ Monitoring of biological cycle and ecology of species *Cameraria ohridella*.
- ✓ Monitoring of species *Cameraria ohridella*.
- ✓ Integrated combating of species *Cameraria ohridella*.

Chapter 4. "Working material and methods"

During the research period for achieving the proposed objectives we used the methods adapted to the concrete conditions of the experimental years and used frequently in the entomology activities. There are presented methods for monitoring the climatic conditions during the period 2015-2018, the adults, the frequency of attack produced by larvae, the flight orientation in the crown of trees, the integrated combating with chemical products with different chemical basis based on: spinosad, chlorpyrifos methyl, abamectin, lambda-cyhalothrin, spirotetramat, diflubenzuron and bioproducts (pheromones) purchased from the Institute of Chemistry "Raluca Ripan" in Cluj Napoca, an autochthonous pheromone variant: 37-atraCAM and three **new pheromone** variants provided for testing.

Chapter 5. "Results and discussions"

5.1. Results regarding climatological analysis of the area during the period 2016-2018

In the analysis of the thermal, pluviometric and hygrometric regime of the Caracal area, the averages for the period 1981-2015 were calculated monthly and multiannually, and for the period 2016-2018 at the decadal, monthly and annual level. The average annual temperature is 12.2°C in 2016, 11.7°C in 2017 compared to 11.1°C during the period 1981-2015. The annual amount of rainfalls is 545.3 mm in 2016; 594.6 mm in 2017 compared to

520.5 mm recorded during the period 1981-2015. The increase in the minimum annual temperature is 8°C in 2016 and 7°C in 2017 compared to the period 1981-2015.

The maximum annual temperature is 17.8°C in 2016, 17.9°C in 2017 compared to 17.5°C during the period 1981-2015.

5.2. Research regarding the external morphology of development stages: larva, nympha, adult of the mining moth

From the research performed on the adult body, it has a length of 2.276 mm and a width of 0.450 mm (figure 1), smaller values than the data in the specialized literature of 2.4-3.0 mm (DAMIAN, 2008). The antennae of the chestnut moth are filiform and 0.986 mm long, and OLTEAN, 2007; DAMIAN, 2008, show that the length of antennae is 2.6-3.2 mm. The length of the front wings is 2.932 mm and that of the rear wings is 2.160 mm. The span of the population of chestnut leaf mining moth in the south-east part of Oltenia is 5.092 mm, it being smaller than the span of 7.3-7.7 mm, stated by DAMIAN, 2008 and the wingspan of 6-7 mm is supported by RÁCOSY AND RUICĂNESCU, 1998. The larva has a length of 3.222-3.980 mm and a width of 0.576-0.567 mm. The body is flattened in the dorsal-ventral side, sharpened in the posterior side, with ornamentation at the level of the last abdominal segment and the last pair of pseudopods. The dimensions obtained from the measurements of the development stages of the mining moth captured in Constantin Poroineanu Caracal Park are smaller than those mentioned in the specialized literature.



Fig. 1. *Cameraria ohridella* Deschka-Dimić, adult - ventral, endowed with all the appendices, including spirotrompe

5.3. Monitoring of biological cycle and ecology of species *Cameraria ohridella* Deschka-Dimić

5.3.1 Biological cycle of species *Cameraria ohridella* Deschka-Dimić

In Caracal area, the biological cycle was influenced by the evolution of climatic conditions recorded during the research period. Thus, in 2016, the chestnut leaf mining moth developed 4 complete generations (the fifth generation is partial, the nymphs enter the hiemal diapause during the period 05.10-25.04.2017). The biological activity of the species was carried out during the period April 28 - October 13 2016, amounting 160 days. From the analysis of the data regarding the biological activity of the chestnut leaf mining moth in 2017, it can be seen that it comprised the period April 25 - September 17, for 145 days, it being shorter with 15 days compared to 2016, the insect developing only 3 complete annual generations (the fourth generation is partial) due to the climatic conditions recorded during the biological activity period (figure 2.) In 2018 the mining moth presented up to the current moment of research a total of three complete generations.

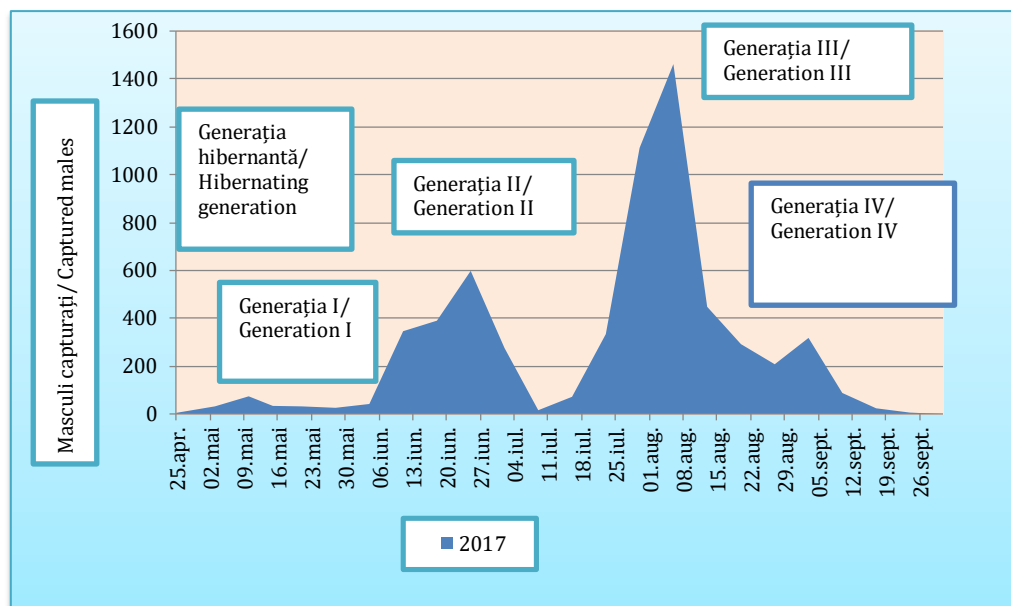


Fig. 2. Biological cycle of species *Cameraria ohridella* Deschka-Dimić (Caracal, 2017)

5.3.2. Flight dynamics of adult *Cameraria ohridella* Deschka-Dimić

During the entire flight period of adults of species *Cameraria ohridella* Deschka-Dimić in 2016, 8438 males were captured with the aid of pheromone baits traps.

The flight of adult chestnut moth in 2017 was staggered during the period April 25 - September 26 for a period of 154 days.

2018 brings major changes related to flight and capturing of males. The captures in this year were very low, namely 1033 males during the entire flight period of the species April 22 - August 31, due to the evolution of microclimate conditions.

5.3.3. Obtained results regarding the ecology of species *Cameraria ohridella* Deschka-Dimić

5.3.3.1. Obtained results regarding the action of climatic conditions on species *Cameraria ohridella* Deschka-Dimić

During the year 2016, the correlation between the duration of generations and the average temperature is moderately positive, the one between the duration of generations and rainfalls is poorly positive, and the one between the duration of generations and atmospheric humidity is weakly negative. During the year 2017, the correlation between the duration of generations and the average temperature is moderately negative, the one between the duration of generations and rainfalls is strongly positive and the one between the duration of generations and atmospheric humidity is very positive.

5.4. Results regarding the monitoring of species *Cameraria ohridella* Deschka-Dimić

5.4.1. Monitoring of adult *Cameraria ohridella* Deschka-Dimić during the period 2016-2018

The analysis of results concerning the dynamics of decadal captures obtained during the period April-October, reveals that the population presents during the year 2016 four maximums, three maximums in 2017 and three maximums in 2018 (figure 3). The first numerical growth of the population occurs at the end of May, the second increase is recorded in the second decade of July (2016), the third increase is recorded in August (second decade), and the fourth increase is recorded in September the third decade (2016). The maximum number of captured individuals was recorded in 2016 at the end of July, compared with 2017 when it was recorded in the first decade of August. In 2018, the population decreased considerably due to the unfavorable climatic conditions, heavy rainfalls in July of 110 mm and atmospheric humidity of 72.8%. These conditions allowed an engage in flight of a reduced number of males.

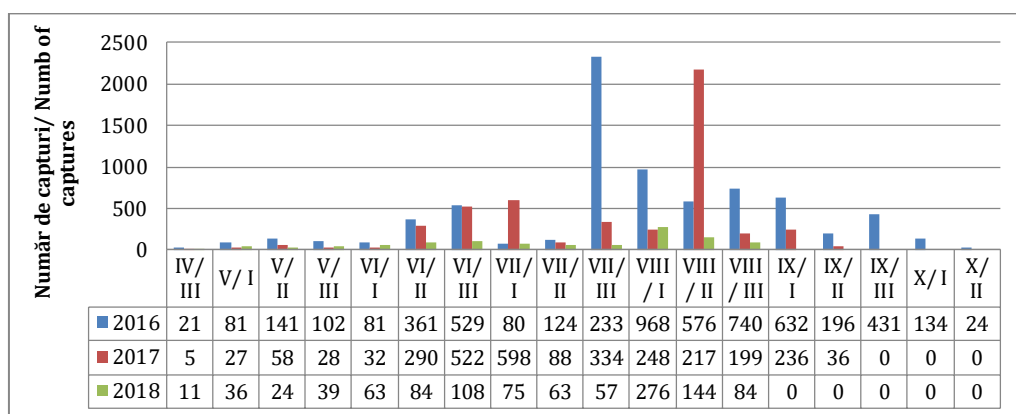


Fig. 3. Decadal abundance of the population of chestnut leaf mining moth during the period 2016-2018, Caracal

5.4.2. Results regarding the determining of flight orientation in the crown of trees

In 2017, the most significant level of captures recorded in the crown of trees was to the south (463 males), followed by the captures recorded on the eastern traps (434 males) and by the captures recorded from the pheromone traps placed to the west (314 males). The smallest number of captures of 250 specimens was recorded to the captures from the traps placed to the north.

Year 2018 brings major changes related to the very low captures for all traps irrespective of exposure: 203 males on the eastern traps, 106 males on the traps oriented to the west and 106 males on the traps oriented to the north. During this year, the number of captured males also indicates the preference of the species for the southern exposure (229 males), to the detriment of the other.

5.4.3. Monitoring species *Cameraria ohridella* Deschka-Dimić based on the frequency of attack produced by larvae during the period 2017- 2018

On the ornamental chestnut trees in Constantin Poroineanu Park, the attack produced by larvae of the chestnut leaf mining moth was monitored by making observations and determinations in the first decade of July and the second decade of August. At the observation in July on the leaves with eastern exposure, the chestnut leaf mining moth developed an average number of 3.3 mines/leaf (2017), 1.6 mines/leaf (2018) and 0.47 mines/foliolate (2017), 0.22 mines/foliolate (2018). Since this date it grew slowly, so that the observation in August reached an average number of 3.6 mines/leaf (2017), 1.9 mines/leaf (2018) and 0.07 mines/foliolate (2017), 0.27 mines/foliolate (2018).

At the first observation on the leaves with western exposure, the average number of mines was 1.9 mines/leaf (2017), 1.4 mines/leaf (2018) and 0.27 mines/foliolate (2017), 0.20 mines/foliolate (2018). In August, a fast increase was reported in the final assessment, with an average number of 3.1 mines/leaf (2017), 1.8 mines/leaf (2018) and 0.4 mines/foliolate (2017), 0.26 mines/foliolate (2018).

On the leaves with northern exposure, at the observation in July were recorded values of 1.8 mines/leaf (2017), 0.9 mines/leaf (2018) and 0.26 mines/foliolate (2017), 0.13 mines/foliolate (2018). Until the following observation of the number of mines/leaf/foliolate it reached a number of 2.5 mines/leaf (2017), 1.2 mines/leaf (2018) and 0.36 mines/foliolate (2017), 0.17 mines/foliolate (2018). In July the number of mines on leaves with southern exposure was of 4.1 mines/leaf (2017), 2.3 mines/leaf (2018) and 0.58 mines/foliolate (2017), 0.33 mines/foliolate (2018) and reached in August a number of 5.1 mines/leaf (2017), 3.5 mines/leaf (2018) and 0.72 mines/foliolate (2017), 0.5 mines/leaf (2018). It can

be concluded that the southern exposure is preferred by the larvae, therefore the highest values of the average number of mines/leaf/foliolate was reached on these leaves, indicator which increased over the time interval between two observations.

5.5. Results regarding the integrated combating of species *Cameraria ohridella* Deschka-Dimić

5.5.1. Obtained results in testing insecticides for combating species *Cameraria ohridella* Deschka-Dimić

From the analysis of data obtained during the period 2016-2018 there were found obvious differences between the treatments applied for combating the pest with both systemic, contact products and treatments applied with biological products. The highest efficacy is obtained with the product Vertimec 1.8 EC, followed by Movento 100 SC, Pyrinex M22, Dimilin 48 SC. The product Laser 240 EC recorded the lowest efficacy (table 1).

Table 1.

Efficacy of the method of capturing adult (males) mining moth on the trees treated with insecticides (Caracal, 2016-2018)

No.	Used insecticide (Active substance)	Conc. %	Efficacy %		
			2016	2017	2018
1.	Laser 240 EC 240 EC (spinosad)	0.04	70.09	78.26	71.8
2.	Pyrinex M22 (chlorpyrifos methyl)	0.15	84.25	88.40	89.87
3.	Vertimec 1,8 EC (abamectin)	0.07	93.55	97.62	96.02
4.	Karate Zeon (lambda-cyhalothrin)	0.015	80.67	85.77	78.31
5.	Movento 100 SC (spirotetramat)	0.15	88.55	97.00	92.28
6.	Dimilin 48 SC (diflubenzuron)	0.03	91.86	95.78	93.97

VII

5.5.2. Obtained results regarding the mass capturing of males with the aid of pheromone traps depending of the efficacy of used insecticide

During the period 2016-2018 the evolution of capturing values in each experimental variant was monitored. By comparing the three experimental years, there is a reduction in captures from one year to another, results which deserve to be taken into account when establishing strategies for combating the pest (table 2).

5.5.3. Obtained results regarding the biocombating of species *Cameraria ohridella* Deschka-Dimić with the aid of attracting pheromones

The research performed during the period 2016-2018 demonstrated that under natural conditions, the maximum efficacy of capturing adults was recorded by the four pheromones in the interval August 01-07 2016, July 24-31 2017, July 15-22 2018 (figure 4).

Table 2.

Efficacy of some insecticides in combating species, expressed by the number of captured adults, Caracal, (2016-2018)

No.	Used insecticide (Active substance)	Conc. %	Number of captured adults/Year			
			2016	2017	2018	2016 -2018
1.	Laser 240 EC 240 EC (spinosad)	0.04	1343	734	316	2393
2.	Pyrinex M22 (chlorpyrifos methyl)	0.15	816	578	263	1657
3.	Vertimec 1,8 EC (abamectin)	0.07	481	423	146	1050
4.	Karate Zeon (lambda-cyhalothrin)	0.015	964	714	312	1990
5.	Movento 100 SC (spirotetramat)	0.15	472	526	252	1250
6.	Dimilin 48 SC (diflubenzuron)	0.03	469	431	152	1052
7.	Untreated control sample	-	2792	1531	474	4797
Total/year			7337	4937	1915	14189

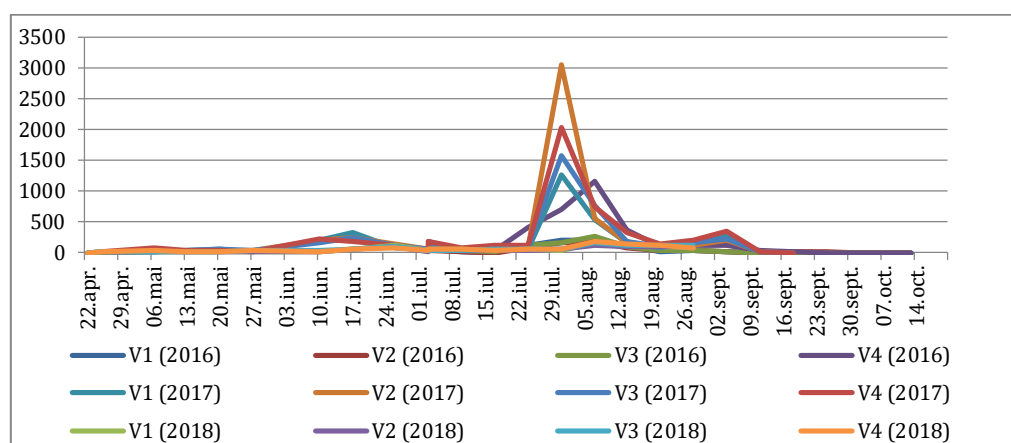


Fig. 4. Variation of captures at *Cameraria ohridella* Deschka-Dimić, Caracal, 2016-2018

5.5.4. Results regarding the combating of species *Cameraria ohridella* Deschka-Dimić with the aid of attracting pheromones depending on the evolution of climatic factors

From the data recorded for 2016, under the climatic conditions: (average temperature 12.2°C, maximum temperature 17.8°C, minimum temperature 7.7°C, rainfalls 545.3 mm and atmospheric humidity 74.4%), it results that the number of captures achieved by the four pheromones varied depending on the attractiveness of the pheromone: 3859 males/Pheromone 4; 1037 males/Pheromone 3; 925 males/Pheromone 1; 812 males/Pheromone 2. The level of captures was influenced in 2017 by the climatic conditions (temperature: average 11.7°C, maximum 17.9°C, minimum 6.7°C, rainfalls 594.5 mm, atmospheric humidity 73.1%), as follows: Pheromone 2: 5303 males; Pheromone 4: 5085 males; Pheromone 3: 4129 males; Pheromone 1: 3575 males. In 2018 the level of

captures was influenced by the climatic conditions (temperature: average 21.7°C, maximum 28.6°C, minimum 15.9°C, rainfalls 282.2 mm, atmospheric humidity 68.5% as follows: Pheromone 4: 1091 males, Pheromone 1: 1030 males, Pheromone 3: 1102 males, Pheromone 2: 920 males (figure 5).

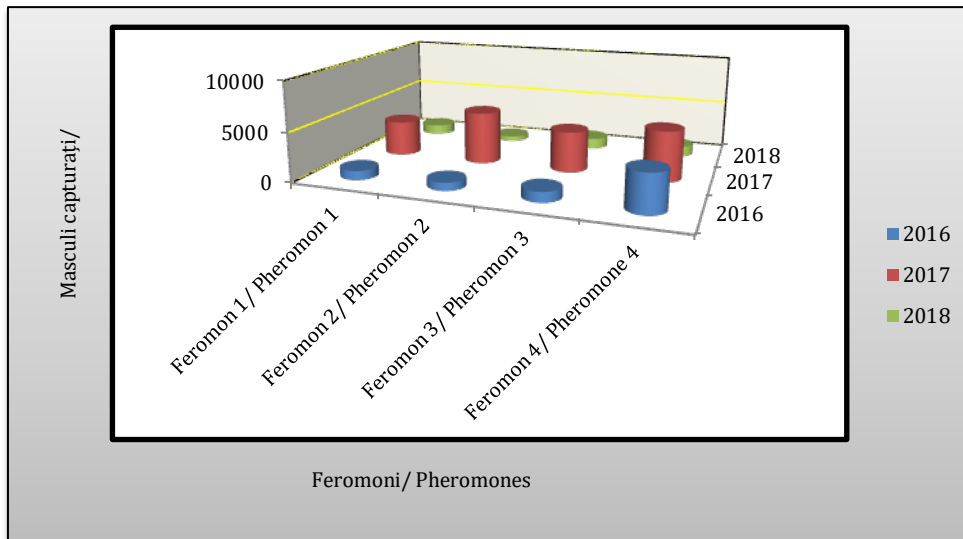


Fig. 5. Captures of *Cameraria ohridella* Deschka-Dimić achieved by the tested pheromones under the climatic conditions in Constantin Poroineanu Caracal Park, period 2016-2018

The average temperature significantly influenced positively ($r = 0.80$) the number of adults captured by pheromone 3, and the rainfalls and atmospheric humidity had a very poor negative influence on the number of captured adults, the correlation factors having values of $r = -0.06$, respectively $r = -0.008$ (figure 6).

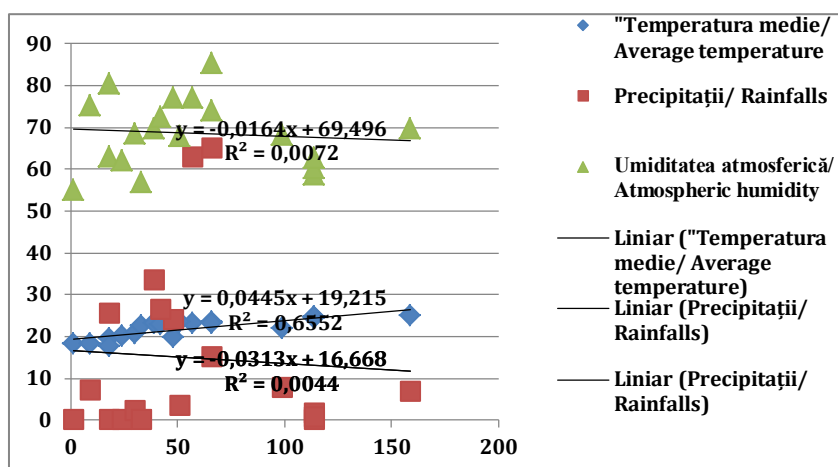


Fig. 6. Regression lines and equations of the number of captures obtained with the aid of pheromone 3 related to climatic conditions
Chapter 6. "Conclusions and recommendations"

Because in urban green spaces in the south-east of Oltenia, species *Cameraria ohridella* Deschka-Dimić is a particularly dangerous pest for the ornamental chestnut, and its continuous monitoring is mandatory. In order to reduce the attack produced by this pest, it is recommended to promote biological methods by performing research to improve the functional parameters for the sexually attractive pheromones used in the ornamental chestnut leaf mining moth.

Comparing the attractiveness of the 3 *new pheromone formulations* over the three experimental years, it was observed that pheromone 3 recorded the most captures in years 2016 and 2018, with attractiveness almost similar to pheromone 2 in 2017. Pheromone 1 was less attractive, followed by pheromone 2. Pheromone 3 proved to be more efficient in capturing and it is due to its chemical composition different from other pheromones.