PhD THESIS

Studies on phytophagous entomofauna from the landscaping of Cluj-Napoca in the context of climate change and pollution levels

(SUMMARY OF Ph.D. THESIS)

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CLUJ-NAPOCA, 2024

INTRODUCTION

Green spaces have many functions, and the most important ones are: improvement of the urban microclimate, reduction of pollution and beneficial effects on the health of the population. Various international organizations show that 50 m^2 of green space per inhabitant would be necessary, and European standards require at least 26 m^2 per inhabitant.

Often the aesthetic value of green spaces is strongly affected by the rich complex of pests. Recently, there has been a continuous increase in the number of newly introduced invasive species. Many of the woody species that are imported for ornamental purposes are infested, and the insects may be in various stages of development on the imported material, often making the infestation difficult to detect. The spread of invasive species is facilitated by the fact that freight transport is constantly increasing, but also due to the decrease in customs controls. The penetration of foreign invasive species is also facilitated by the climate changes.

The management of the protection of landscaping is a complex activity that is primarily based on a continuous monitoring of phytophagous entomofauna that is strongly influenced by the urban environment. That's why finding solutions to keep pests of dendrofloric plants below the economic threshold of damage (PED) is a concern of specialists in the field of plant protection.

1. Structure of the doctoral thesis

The doctoral thesis includes a total of 130 pages and was structured in two parts:

- Current state of knowledge - part I (40 pages)
- Personal contribution – part II (90 pages)

The second part totals a number of 90 pages, structured in 6 chapters, which describe the objectives, the environment used in the experiments, materials and methods, results obtained, discussions, conclusions and recommendations as well as the originality and innovative contribution of the thesis. The thesis contains a total number of 22 tables, 43 figures, 294 bibliographical titles and 3 sites.

2. Research objectives

The purpose of the research carried out during the doctoral period was to study the harmful species, especially the invasive ones, which affect the aesthetic value of the plants in the Cluj landscaping in the period 2020-2022.

The research objectives of this thesis are:

1. Bioecology of the species *Obolodiplosis robiniae* Haldeman;
2. Bioecology of black locust mining moths;
3. Monitoring of the *Oxycarenus lavaterae* Fabricius species;
4. Structure of lime mite species;
5. Monitoring of the species *Metcalfa pruinosa* Say;
6. Monitoring the species *Pulvinaria floccifera* Westw.

3. Material and methods

1. Bioecology of the species *Obolodiplosis robiniae* Haldeman

Since in 2019 the species *Obolodiplosis robiniae* Haldeman was reported in Cluj-Napoca, in 2020 and 2021 it was monitored. The studies were carried out in two locations in the city of Cluj-Napoca: at the black locust in the "Simion Bărnuţiu" Central Park and at the black locust in some street alignments. Then, 50 attacked leaves were randomly harvested in 3 repetitions from each location, only from the attacked trees, and their analysis was performed in the laboratory. The number of leaflets attacked on each leaf, the number of galls/leaflet and the number of larvae/gall, were determined.

2. Bioecology of black locust mining moths

During the years 2020-2021, the frequency of the attack produced by the two black locust mining moths, *Macrosaccus* (*Phyllonorycter*) *robiniella* Clemens and *Parectopa robiniella* Clemens, was monitored, as well as the number of mines they develop on an black locust leaf . The monitoring of these elements was done three times a year, in order to compare and indicate as correctly as possible the trends that appear in the evolution of the two monitored quantitative parameters. The first collection was carried out between 07.07 - 07.15; the second collection in the period 12.08 - 19.08, and the last in the period 10.09 - 17.09. Because, visually, we found that the frequency of attacked leaves is relatively low, for a better assessment of the attack frequency, at each collection, 10 trees were randomly chosen from which 100 leaves were collected, a total of 1000 leaves. The leaves taken from the field were analyzed in the laboratory.

To establish the biological cycle of the species *Parectopa robiniella*, samples were taken annually from the attacked leaves (200 leaves for each observation), starting from the second half of May and until September, leaves that were analyzed in the laboratory and the developmental stages of the studied species were followed.

The flight curve of the adults was studied based on the captures made by the traps with sexual attractant pheromones and the visual white traps.

3. Monitoring *Oxycarenus lavaterae* Fabricius

During April-May 2021-2022 on the trees of *Tilia cordata* Mill., *Tilia platyphyllos* Scop. and *Tilia tomentosa* Moench located on the street alignments in Cluj-Napoca, we proceeded to verify the existence of hibernating colonies of the species *Oxycarenus lavaterae* Fabricius on these trees. We determined the frequency of infested trees, the number of existing hibernating colonies on a tree and the shape of the colonies. For the colonies sampled, in the laboratory we analyzed the number of specimens in a colony. I also followed the period of appearance of each stage of development.

4. The structure of mite species on linden trees

In the period 2020-2021, the mites that attack the linden were monitored. For linden mite species, 60 trees (20 *Tilia cordata* trees, 20 *Tilia platyphyllos* trees, 20 *Tilia tomentosa* trees) located on street alignments were analyzed. 100 leaves from the base of the canopy of each tree observed were analyzed and the mite species was determined by the shape of the gall. The frequency of attacked leaves out of the total analyzed leaves

was determined. For each mite species, the number of galls developed on a leaf was determined depending on the linden species attacked.

5. Monitoring of the species *Metcalfa pruinosa* Say

The observations were made in the green spaces and on the street alignments in the city. The observations were made annually during the months of May - September. *Metcalfa pruinosa* Say. was identified based on the morphological characteristics of the adults present on the infested plants, as well as the larvae colonies that leave very obvious white filaments at the site of the attack. Based on field observations, the biological cycle of the species was established.

6. Monitoring of the species *Pulvinaria floccifera* Westw.

Observations for this species were carried out on *Ilex aquifolium* L plants. In order to establish the biological cycle, both observations were carried out in the field and branch samples were taken, and in the laboratory the gradation of the different stages of development of the species was established. The number of existing bridges on the analyzed branches was also monitored. To combat this species, three treatments were applied, carried out in correlation with its biological cycle. The insecticides used were: Karate Zeon 0.03% (Lambda-cyhalothrin) and Coragen 0.03% (Chlorantraniliprole)

4. Results and discussions

4.1. Results regarding the bioecology of the species *Obolodiplosis robiniae* Haldeman

In Romania, *Obolodiplosis robiniae* Haldeman was reported in Bucharest in 2007, being recently also reported in Cluj-Napoca. The frequency of attacked leaves of the black locust in the Central Park was 8% in 2020 and 15% in 2021, and in the acacias on the alignments it was 3% in 2020 and 7% in 2021.

In samples of attacked leaves taken randomly from each location, the average number of leaflets per 50 leaves in the Central Park was 985 in 2020 and 932 in 2021 and in alignments of 963 in 2020 and 1010 leaflets in 2021. In 2020 in the Central Park, out of the total analyzed leaflets, 310 presented galls (31.5%), and among those on the alignments, 121 leaflets were attacked (12.6%). From the total of leaflets analyzed in 2021, in the Central Park 373 had galls (40%), and from the alignments 194 leaflets had galls (19.2%).

Analyzing the total number of fouls on the closed leaflets, in 2020 in the Central Park there are 485 fouls on the 310 attacked leaflets, which means an average of 1.6 galls/foliole, and on the alignments on the 121 attacked leaflets there are 162 galls, on average being 1.3 galls/leaflet. The following year, in the Central Park on the 373 leaflets attacked there are 659 gals, which means an average of 1.8 gals/folio, and on the alignments on the 194 leaflets attacked there are 291 gals, on average being 1.5 gall/leaflet.

The number of gales/leaflet was between 1 and 5 gales in the Central Park, and between 1 and 4 gales on the alignments. The leaflets with a single gala represent 53.5%

of the total leaflets attacked in the Central Park, and on alignments 50.4%. The leaflets with two galas are in the proportion of 29.7% in the Central Park and 36.4% in the alignments. The leaflets with three galas are in the proportion of 15.2% in the Central Park and 10.7% in the alignments. Leaflets with four galls are in the proportion of 1.3% in the Central Park and 2.5% in the alignments. Leaflets with five galls were reported only in the Central Park, with a proportion of 0.3%.

The impact on the host plant is also correlated with the number of larvae in a gala. On the material collected in both years, the number of larvae/gala was between 1-5 larvae in the material taken from the Central Park, respectively between 1-3 larvae in the alignments. In both locations and in each year, the number of galls with a single larva was the highest, 58% of the total galls in the Central Park and 72% on the alignments, in 2020, respectively 51% of the total galls in the Central Park and 69% on alignment in 2021. Gales with two larvae were in the proportion of 28% in Central Park and 20% on alignments in 2020 and 25% in Central Park and 19% on alignments in 2021. Three larvae each were in the percentage of 10% of the galas in the Central Park and 8% in those on the alignments in 2020, respectively 18% of the galas in the Central Park and 12% in those on the alignments, in 2021. In the Central Park they there were 3% of galls in 2020 and 5% of galls in 2021 with four larvae and 1% of galls with five larvae in both years.

4.2. Results on the bioecology of black locust mining moths

In 2020, the frequency of leaves attacked by mining moths was 2.3% in July, 4.3% in August, and at the final observation in September, made at the end of the miners' biological cycle, the attack frequency reached 7,1%. Since the two species of mining moths have the same distribution area, they can attack the leaves singly or in a complex. Thus, 5.7% of the leaves were attacked by *Parectopa robiniella* or *Macrosaccus robiniella*, and 1.4% attacked simultaneously by both species.

In 2021 at the first observation, the attack was reported on 2.7% of leaves. At the second observation there was an increase in the attack frequency, this time 5.4% of the leaves were attacked, and at the September observation the attack frequency reached 7.5%. Of the attacked leaves, 5.4% presented the mines of a single species, and 2.1% are simultaneously attacked by both species.

In 2020, the number of mines that the species develop on a leaf has increased from one observation to another, so that at the end of the evolutionary cycle they reached 4.3 min/leaf for *Parectopa robiniella* and 2.4 min/leaf for *Macrosaccus robiniella* when they attacked singly, and when they attacked simultaneously it was 5.5 mines/leaf, of which 2.6 mines/leaf in *Parectopa robiniella* and 2.9 mines/leaf in *Macrosaccus robiniella*.

At the last observation in 2021, the number of mines reached 5.1 mines/leaf in *Parectopa robiniella* and 2.9 mines/leaf in *Macrosaccus robiniella*, and when they attacked simultaneously it was 6.5 mines/leaf, from which 3.8 min/leaf in *Parectopa robiniella* and 2.7 min/leaf in *Macrosaccus robiniella*.

Considering the fact that during the monitoring period of the mining moths of the acacia, *Parectopa robiniella* was the dominant species of this species, we also proceeded to study the biological cycle.

The adults of the hibernating generation (the second generation) resume their activity starting from the first days of June. Immediately after the start of the flight, the females start laying eggs on the black locust leaves.

In the first generation, the incubation period is 11-13 days, and in the second generation, 8-10 days. The minimum duration of development of the larval stage in the first generation was 43-46 days, and in the second generation 34-36 days. The pupal stage lasts 13-16 days.

The adults of the summer generation appear in the first or second decade of August, and their flight activity lasts until the end of August or even September.

4.3. Results regarding the monitoring of the species *Oxycarenus lavaterae* Fabricius

The species *Oxycarenus lavaterae* Fabricius has been reported on all linden species, but the frequency of infested trees is different. In the two years of monitoring the hibernating colonies, we reported them on 5% of the checked trees of *Tilia cordata*, on 3% of the trees of *Tilia tomentosa* and less than 1% on the trees of *Tilia platyphyllos*.

The species was also reported on other plants that were in the vicinity of lime trees, on *Hibiscus syriacus*, *Platanus acerifolia* and *Populus* spp., but the colonies were small, with a small number of specimens.

The size of a colony is between 8 and 18 cm^2, and the number of specimens in a colony in 2021 was between 605 and 2962 individuals, with an average of 1718, and in 2022 between 785 and 3428 individuals, with an average of 2117.

The hibernating adults from the second decade of May, started the migration from the hibernating colonies and spread in the crown of the trees. This activity corresponded to the appearance of flower buds in later lindens, respectively with flowering in earlier species. After a period of additional feeding, copulation and spawning began, and the first larvae were reported in July. From August, the adults of the first generation appeared, and the first hibernation colonies located on the trunks of lime trees were reported on September 15 in 2021, respectively on September 27 in 2022.

4.4. Results regarding the structure of mite species on linden trees

Eriophyes tiliae Pgst. in 2020, it had the highest attack frequency on *Tilia tomentosa* (51%) and the highest attack intensity on *Tilia tomentosa*, this being 15.6%. In this species, the maximum number of galls on an attacked leaf was 109 galls. The lowest attack occurred on *Tilia cordata*, where the attack frequency was 12%, and the intensity was 4.5%. On this species, the maximum number of galls on a leaf was 76 galls.

In 2021, the frequency of the attack produced by *Eriophyes tiliae* Pgst. again had the highest attack frequency on *Tilia tomentosa*, which was 58%, and the attack intensity on this linden species was 17.1%. The maximum number of galls on an attacked leaf was

121 galls. The lowest attack occurred on *Tilia cordata*, where the frequency of the attack was 15%, and the intensity was 8.5%.

Eriophyes rudis Nalep., in 2020, had the highest attack frequency on *Tilia tomenosa*, 29% and attack intensity 11.1%. In this species we also reported 58 galls on one leaf. The lowest attack occurred on *Tilia platyphyllos,* where the attack frequency was 11%, and the intensity was 9.1%. On this species, the maximum number of galls on a leaf was 26 galls. In this species. in 2021 again the highest attack frequency was on *Tilia tomenosa*, of 34% and also on this linden species the highest intensity of attack was also of 14.3%. In this species we also reported 69 galls on one leaf. The lowest attack occurred on *Tilia cordata*, where the attack frequency was 14%, and the intensity was 7.2%. On this species, the maximum number of galls on a leaf was 24 galls.

Eriophyes exilis Nalep. has the lowest numerical population density. Thus, in 2020 the frequency of the attack was between 2% on *Tilia tomentosa* and 5% on *Tilia platyphyllos*, and the intensity of the attack between 2.9-3.9%. In this species, we reported a maximum of 23 galls on a leaf. In 2021, an increase in population density was recorded, but it still remains the species with the lowest numerical population density. Thus, the frequency of the attack was between 4% on *Tilia tomentosa* and 9% on *Tilia platyphyllos*, and the intensity of the attack between 3.7% on *Tilia tomentosa* and 6.3% on *Tilia platyphyllos*. In this species, we reported a maximum of 26 galls on a leaf.

4.5. Results regarding the monitoring of the species *Metcalfa pruinosa* Say

Proceeding to establish the biological cycle, in the investigated locations we found that from hibernating eggs, in 2020 the larvae hatched in the second decade of April, and in 2021 in the last days of April. This difference between the two years is determined by the average monthly temperature of March and April. Larval development lasted around 70 days, so they were reported on the attacked plants until the beginning of July. The adults appeared in July and continued the flight period until mid-September. They deposited their eggs in the cracks of the bark of the host plant, this being the hibernating stage.

The species was found on 35 plant species, which are systematically classified into 19 families. Most of the plants are from the families: Malvaceae, Oleaceae and Sapindaceae, families from which four species were identified. The most attacked plants were: *Acer platanoides, Aesculus hippocastanum, Buxus sempervirens, Fraxinus albicans, Fraxinus excelsior, Ligustrum vulgare, Robinia pseudoacacia* and *Thuja accidentalis*.

4.6. Results regarding the monitoring of the species *Pulvinaria floccifera* Westw.

The monitoring of this species was carried out only on *Ilex aquifolium* L. In 2020 the hibernating larvae resumed their activity in the first half of April, and in 2021 in the last decade of April.

The adults appeared in the first decade of May in 2020, respectively in the second decade of May in 2021.

The clutches deposited by the female in the ovigenic sacs were in June. The number of pons laid on a leaf was between 2-13 pons. On one shoot there were up to 92 pontes.

The new generation larvae appeared in the first half of July, followed by the appearance of the secondary larvae starting in August and the third age larvae appear towards the end of this month and in September. Both a part of the second-instar larvae (which appeared later) and the third-instar larvae enter hiemal diapause.

To combat this pest, three treatments were applied annually. The first treatment was applied at the end of April with Karate Zeon 0.03%. The second treatment was carried out after laying the eggs, in July, with Coragen 0.03%. The third treatment was applied in August with Coragen 0.03% + Karate Zeon 0.03%. This chemical control scheme has given great results, being 100% effective.

The phytosanitary status of the ornamental species in Cluj-Napoca in general is particularly good. The attacks caused by some pests are sporadic and with a very different distribution from one area to another.

In *Buxus sempervirens* the most frequent pests are: *Cydalima perspectalis* and *Monarthropalpus buxi*. For *Aesculus hippocastanum* the main pest is *Cameraria ohridella*. An attack by *Macrosiphum rosae* was reported on roses. In shoots, the attack frequency is between 11% in 2021 and 27% in 2021. In floral buds, the attack frequency is between 8% in 2020 and 15% in 2021. On the alleys between the residential quarters of Cluj there are numerous hedges that are attacked by different species of aphids: *Aphis spiraephaga*, *Aphis fabae* and *Aphis spiraecola*. The frequency of attacked shoots is between 59-98%.

5. Conclusions and recommendations

The species *Obolodiplosis robiniae* was recently reported in Cluj-Napoca. The frequency of attacked leaves of acacias in parks was 8% in 2020, respectively 15% in 2021, and for acacias on alignments it was 3% in 2020 and 7% in 2021.

The average number of gals/leaflet in the park was 1.6 gals in 2020 and 1.8 gals in 2021, and on alignments 1.3 gals in 2020 and 1.5 gals in 2021.

At the end of the biological cycle of the acacia miners, in 2020 the attack frequency reached 7.1% and in 2021 7.5%.

In 2020, the flight of the adults of the hibernating generation started on June 5, and in 2021 on June 1, and the flight activity was extended until June.

Incubation is 11-13 days for the first generation and 8-10 days for the second generation; the minimum duration of the larval stage was 43-46 days for the first generation and 34-36 days for the second generation; the pupal stage lasts 13-16 days.

The first adults of the summer generation, in 2020, started flying on August 17, and in 2021 on August 10.

During the two years of monitoring the hibernating colonies of *Oxycarenus lavaterae*, we reported them on 5% of the checked trees of *Tilia cordata*, on 3% of the trees of *Tilia tomentosa* and less than 1% on the trees of *Tilia platyphyllos*.

The size of a colony is between 8 and 18 cm^2, and the number of specimens in a colony in 2021 was between 605 and 2962 individuals, with an average of 1718, and in 2022 between 785 and 3428 individuals, with an average of 2117.

Eriophyes tiliae. had the highest frequency of attack on *Tilia tomentosa*, 15.6% in 2020, respectively 17.1% in 2021; *Eriophyes rudis*, had the highest frequency of attack on *Tilia tomenosa*, 29% in 2020 and 34% in 2021; *Eriophyes exilis* had the highest attack frequency on *Tilia platyphyllos*, 5% in 2020, and 9% in 2021.

The species *Metcalfa pruinosa* Say was found on 35 plant species, systematically in 19 families. The most attacked plants were: *Acer platanoides, Aesculus hippocastanum, Buxus sempervirens, Fraxinus albicans, Fraxinus excelsior, Ligustrum vulgare, Robinia pseudoacacia* and *Thuja accidentalis*.

A relatively large attack was produced by the species: *Cydalima perspectalis. Monarthropalpus buxi, Cameraria ohridella, Macrosiphum rosae, Aphis spiraephaga* and *Aphis spiraecola*.

Recommendations

In order to maintain an appropriate phytosanitary situation of the plants in the green spaces, continuous monitoring of the pest species of these plants is required.

Since the spread of the species *Obolodiplosis robiniae* Haldeman, as well as other pests, is most often done through the planting material, it is necessary that the nursery material used for new plantings is free from it.

For the species that attack the plants in the hedges, it is recommended to clean them immediately after the installation of the pests to reduce the numerical density of the populations of these species.

Raising the awareness of the owners' associations regarding the importance of maintaining the plants in their green spaces, in order to maintain the aesthetic value of the plants.

6. Originality and innovative contributions of the thesis

The presence of the species *Obolodiplosis robiniae* Haldeman was reported for the first time on acacias in Cluj-Napoca.

The first studies on the bioecology of the species *Obolodiplosis robiniae* Haldeman were carried out.

It was found that in the Cluj area the dominant species among the acacia mining moths is *Parectopa robiniella* Clemens, whose bioecology was also studied.

The presence of the species *Oxycarenus lavaterae* Fabricius was reported on the lime trees in the green spaces of the municipality and the frequency of the attack was established depending on the host species.

The bioecology of the species *Oxycarenus lavaterae* was studied and images were made that can contribute as iconographic material for specialized literature.

The species structure of linden eriophyid mites and their preference for linden species was established.