PhD THESIS

Evaluation of monitoring methods to determine the spread and bioecology of invasive insect species

(SUMMARY OF THE DOCTORAL THESIS)

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INTRODUCTION

In the context of globalization and the intensification of international trade, the emergence and spread of invasive insect species have become a major concern in the field of plant protection and biodiversity conservation. Species such as *Drosophila suzukii* and *Thrips setosus* represent significant threats to agricultural and horticultural crops, due to their high ecological adaptability, rapid colonization capacity and difficulty in control in agricultural systems.

Invasion is a normal ecological process, but it can be stimulated by human activities through the transport and trade of different plant materials (Kenis *et al.,* 2008; Liebhold *et al.,* 2012; Eschen *et al.,* 2015). This often allows pest species to cross natural barriers that limit their dispersal (Levine & D'Antonio, 2003; Kollár, 2014).

In Europe, most invasive alien insect species have been introduced accidentally, and a small number of species have been intentionally introduced, most of them for biological control. Climate change is a determining factor in the ecological remodeling of European ecosystems. Global warming directly influences critical ecological parameters, such as survival rate, reproductive success, ontogenetic development rate and dispersal capacity. These changes create favorable conditions for the expansion of the range of these species such as *Drosophila suzukii* and *Thrips setosus*, which can destabilize native communities, affecting the structure and functioning of local ecosystems. At the same time, the interaction between climate dynamics and the mobility of these species poses major challenges for biodiversity conservation and management strategies (Hrubík & Kollár, 2007; Roques, 2010).

Due to the absence of native enemies and altered ecological conditions, accidentally introduced pest species can suddenly increase their population density in the new area (Hrubík & Kollár, 2007). In order to develop effective control and prevention strategies, detailed knowledge of the bioecology of these species. In this regard, monitoring methods play an important role, providing valuable data on population dynamics, habitat preferences and periods of activity.

STRUCTURE OF THE DOCTORAL THESIS

The doctoral thesis entitled "**Evaluation of monitoring methods to determine the spread and bioecology of invasive insect species**" is structured into two main parts, comprising a total of 132 pages, which include 9 chapters, 71 figures, 7 tables, and 183 bibliographic references. **The first part** of the thesis discusses the current state of knowledge related to the specific research topic and is structured into two chapters totaling 22 pages. **The second part** presents the original contribution based on the results obtained during the experimental research period, covering 100 pages and structured into seven chapters.

CURRENT STUDY OF KNOWLEDGE:

Chapter 1, entitled **The Importance of Invasive Species**, summarizes information on the current status of invasive species; measures to prevent and introduce invasive species and the current status of methods of control against invasive pests (mechanical, biological and chemical methods).

Chapter 2, entitled **Invasive Species and Arthropod Biodiversity**, includes aspects related to two species that were studied, *Drosophila suzukii* and *Thrips setosus*, for which the origin and spread, systematic classification, description of the external morphology of all stages of development, their biological cycle, host plants and mode of attack are presented. This chapter also presents the importance of arthropod biodiversity and biodiversity indicators.

PERSONAL CONTRIBUTION:

Chapter 3 entitled Research purpose and objectives sets out the main purpose of the doctoral thesis, along with the objectives established to achieve it.

Chapter 4 entitled Description of the experimental area provides information on the particularities of the natural environment of the research.

Chapter 5 entitled Testing of food baits in monitoring the *Drosophila suzukii* species, presents the results obtained from the research carried out in the period 2019–2021 on the *Drosophila suzukii* species which allowed the evaluation of the attractiveness effectiveness of three food baits in four ecosystems.

Chapter 6 entitled Monitoring the *Thrips setosus* population using colored panels and the Berlese-Tullgren method presents the results obtained from the research carried out in the period 2020–2021 on the monitoring of the *Thrips setosus* species with traps of different colors and by the Berlese-Tullgren method on three varieties ("Hot Red", "Caipirinha", "Early Blue") in a *Hydrangea macrophylla* crop.

Chapter 7 entitled Monitoring insect populations with the suction trap presents the results obtained on the effectiveness of this method in assessing biodiversity in an agroecosystem.

Chapter 8 entitled Conclusions and recommendations, presents well-supported conclusions drawn from the research, along with recommendations based on its results.

Chapter 9 entitled Originality and innovative contributions of the thesis, presents the innovative aspects of the personal contribution in relation to the topic studied.

RESEARCH PURPOSE AND OBJECTIVES

The purpose of this doctoral thesis is to establish methods for monitoring pest species in different ecosystems, which would facilitate their detection, as well as the assessment of biodiversity in an ecosystem. The objectives established in this work

were formulated in a coherent and targeted manner, so as to contribute to achieving the general purpose of the research.

The main objectives were the following:

- Studying the distribution of the *Drosophila suzukii* population depending on the type of agricultural ecosystem and the solutions used in traps, comparing the efficiency of the attraction methods in the monitored locations.
- Determining the differences in the capture of individuals of different sexes, analyzing whether there are significant variations in the attraction of males and females depending on the habitat and the composition of the food bait.
- Testing the attractiveness of sticky ponours of different colors: green, red-purple, pink, white and fluorescent yellow, in the monitoring of the *Thrips setosus species.*
- Testing the Berlese-Tullgren method with red light in the monitoring of the Thrips setosus species.
- Identifying the main dominant taxonomic units in the targeted agricultural area collected with the suction device.
- Establishing possible correlations between the activity of zoophagous insects and plant protection practices.
- Calculation of biodiversity indices: Shannon–Wiener (H'), Margalef and Simpson (D), for the collected entomofauna.

1st STUDY - Testing of food baits in monitoring the *Drosophila suzukii* species

Introduction: In the period 2019-2021, a study was initiated at two locations in North Rhine-Westphalia: one near Köln and the other in Meckenheim, Germany. In the Köln area, the study was carried out at three sub-locations: a blueberry plantation, a composting area for plant residues from the experimental fields of the Landwirtschaftskammer NRW and in a tree plantation. In Meckenheim, a cherry orchard was included.

The aim to contribute to deepening knowledge on the behavior, distribution and effective methods of monitoring the invasive species Drosophila suzukii in agroecosystems specific to the North Rhine-Westphalia region, Germany, with the objective of optimizing the method of capturing *Drosophila suzukii* individuals by using food traps specially designed at the Raluca Ripan Institute of Chemistry in Cluj.

Materials and methods: The food baits were formulated in three experimental variants, each with a different food composition:

- Variant 1: contains 75% Răureni apple cider vinegar, with an acetic acid concentration of 50g/L + 25% semi-dry Merlot red wine + a drop of apple-flavored dishwashing detergent, according to the study conducted by (Landolt *et al.,* 2012).

- Variant 2: in this mixture, exclusively Răureni apple cider vinegar was used without the addition of wine. To improve the attractiveness and properties of the solution, a teaspoon of brown sugar was added. In addition, to facilitate the release of tension in the solution, a drop of apple-flavored dishwashing detergent was included. This formulation is based on previous research by Burrack *et al.,* 2015.
- Variant 3: This variant uses a balanced ratio of 50% semi-dry Merlot red wine and 50% Răureni apple cider vinegar. To improve the effectiveness of the mixture, a drop of apple-flavored dishwashing detergent was added. The formulation was inspired by the research of (Grassi *et al.,* 2014).

The containers in which the food baits were placed were of two types: the first type was transparent containers, equipped with a yellow lid (Landolt *et al.,* 2012); the second type of traps was colored in two distinct shades. The lower part of the container was treated with a green pigment, while the upper part was characterized by a reddish-violet shade, which extended to the lid, which had a yellow color (Tang & Guo, 2001; Yamaguchi *et al.,* 2010; Kelber *et al.,* 2013; Paulk *et al.,* 2013; Little *et al.,* 2019).

These traps were placed at a height of approximately 1.0 - 1.5 m above the ground. A distance of 20 m was also maintained between traps, to avoid overlapping attraction areas and to obtain a representative sample from each location. The traps were replaced weekly, following a systematic rotation, designed to ensure the efficiency of the experimental method and prevent the accumulation of external factors that could influence the results.

Results and conclusions:

During the monitoring period, a well-defined seasonal evolution of the *Drosophila suzukii* population was observed, with significant variations influenced by climatic and biological conditions, especially by the host plant.

Each year, the flight began in April, with reduced catches until May. The maximum of the flight curve, depending on the year and location, was reported in July, August and September.

In 2019, 63,396 specimens were captured, of which: 5315 in the blueberry crop (8.4% of the annual total), 18669 in the composting area (29.4%) and 39412 in the cherry orchard (62.2%). Only colorless traps were used.

In 2020, colorless and colored traps captured 108100 specimens, of which: 7727 in the blueberry crop (7.1% of the annual total), 16143 in the composting area (14.9%), 65158 in the cherry plantation (60.3%) and 19072 in the fruit orchard (17.6%).

In 2021, 29439 specimens were captured on colorless and colored traps, of which: 2484 in the blueberry crop (8.4% of the annual total), 2862 in the composting

area (9.7%), 11114 in the cherry orchard (37.9%) and 12979 in the fruit orchard (44.1%).

In the three years of the study, 200935 specimens were captured in the four monitored locations, of which: 15526 in the blueberry crop (7.71% of the total catches), 37674 in the composting area (18.69%), 115684 in the cherry orchard (57.4%), and 32051 in the fruit orchard (15.95%).

The difference between the number of catches made depending on the location is statistically ensured.

Depending on the food bait variant, out of the total catches made in the three years, 71757 specimens were in V1 (35.7% of the total catches), 43053 specimens in V2 (21.4%) and 86125 specimens in V3 (42.9%).

On colorless traps, 139214 specimens were captured (69.28% of the total catches), of which: 11041 in the blueberry crop, 27761 from the composting area, 83227 from the cherry plantation, and 17185 from the tree plantation.

On colored traps, 61721 specimens were captured (30.72% of the total catches), of which: 4485 in the blueberry crop, 9916 from the composting area, 32454 from the cherry plantation, and 14866 from the tree plantation.

Of the specimens collected, 99397 are females (49.32% of the total catches), and 102141 are males (50.68% of the total catches).

2nd STUDY – Monitoring the *Thrips setosus* population using colored panels and the Berlese-Tullgren method

Introduction: *Thrips setosus* is classified as a polyphagous insect, as it has been observed to feed on a wide variety of host plants (Vierbergen & Loomans, 2016; Pijnakker et al., 2019).

Thrips setosus has been identified as a significant pest in the context of commercial hydrangeas, mainly due to its specific feeding behavior. This pest is known to cause considerable damage by puncturing plant cells and extracting their contents, leading to visible signs of stress, such as discoloration, silvery scars, deformations and a reduction in plant vigor. The economic implications of this damage can be significant, affecting both the production and aesthetic quality of hydrangeas, which are highly valued in the ornamental plant industry. Infestations with *Thrips setosus* usually generate the following symptoms: plant growth is malformed, silvery scars appear in areas with necrotic cells, white streaks and green-black fecal spots on leaves and flowers (Pijnakker et al., 2019).

The aim

Evaluation of the efficiency of colored panels in detecting and estimating the population density of *Thrips setosus* in hydrangea culture, by analyzing the seasonal and behavioral variations of thrips depending on the color of the panels and the monitored varieties.

Testing the Berlese-Tullgren method with red light as a complementary monitoring tool for extracting *Thrips setosus* from plant material, with the aim of comparing the accuracy and sensitivity of this method in relation to the catches obtained on colored panels.

Materials and methods:

The experiment was carried out in the greenhouse of the Köln Chamber of Agriculture, covering an area of approximately 300 m^2. The study tested the effectiveness of colored panels in monitoring thrips populations. The panels were hand-painted in five distinct colors: green, purple-red, pink, white and fluorescent yellow. They were 5 cm × 10 cm in size.

To test the functionality of the colored panels, three varieties of hydrangea "Hot Red", "Caipirinha" and "Early Blue" were used.

The traps were checked weekly with a magnifying glass, and *Thrips setosus* individuals were counted separately for each sex and for each side of the panel. The position of the panels in the experimental space was changed weekly to avoid influences related to the local microclimate or variable light exposure.

To evaluate the optical characteristics of the colored panels used in the experiment, reflectance measurements were performed in the UV-VIS-NIR regions. These were performed using the HR2000+ fiber optic spectrometer, which operates in the spectral range of 200–1100 nm and provides an optical resolution of 6.8 nm.

To obtain a comprehensive understanding of the developmental trends of the *Thrips setosus* population, the Berlese-Tullgren technique was used (Sapkota *et al.,* 2012). This method is particularly effective for collecting larvae.

Results and conclusions:

In the two years of monitoring the catches of *Thrips setosus* from the *Hydrangea macrophylla* crop, 11461 specimens were captured, of which 4525 in 2020, representing 39.5% of the total catches and 6936 specimens in 2021, representing 60.5% of the total catches.

Depending on the variety of *Hydrangea macrophylla,* 3799 specimens were on the "Hot Red" variety, representing 33.1%; 4272 specimens on the "Caipirinha" variety, representing 37.3%; 3390 specimens on the "Early Blue" variety, representing 29.6%.

In each year and for each variety, the maximum catches were reported in June. In this month there were 3776 specimens, representing 33% of the total catches in the two years.

There are significant differences between the colored panels in terms of catches. In the two years and across the three varieties, the fluorescent yellow panels made 7058 catches (representing 62% of the total catches), the green panels had 2457 specimens (representing 21%), the white panels had 1540 catches (representing 13%).

On the pink and red-purple panels there was the lowest number of catches, 406 specimens, representing only 4% of the total catches.

In both years, both at the monitored variety level and depending on the panel color, the percentage of females is higher, except for 2020, when on the yellow panels the percentage of males was higher.

Through the Berlese-Tullgren technique in the two experimental years, 7945 specimens were captured, of which 3431 in 2020 (representing 43% of the total), and in 2021 4514 specimens were captured (representing 57% of the total).

On *Hydrangea macrophylla* leaves in 2020 using the Berlese-Tullgren technique, 1370 specimens were collected, of which 832 larvae (61% of the total) and 538 adults (39% of the total), and in 2021 there were 2075 specimens.

On *Hydrangea flowers* in 2020 using the Berlese-Tullgren technique, 2061 specimens were collected, and in 2021 there were 2439 specimens.

3rd STUDY – Monitoring insect populations with the suction trap

Introduction: In the period 2020–2021, a detailed entomological investigation was carried out in the Nordrhein-Westfalen (NRW) Region, Köln, Germany, in an experimental agroecosystem, characterized by polyculture and annual application of herbicides and insecticides.

In the context of climate change, insects play an essential role in the functioning and maintenance of the ecological balance of many ecosystems. They represent an essential component of this biodiversity, having an active role in pollination, biological control, decomposition of organic matter and maintaining the trophic balance. Also, an understanding of the dynamics of insect populations is becoming increasingly vital for the development of sustainable management strategies for biological resources.

The aim is to investigate the biodiversity of insect populations in temperate agrosystems using suction traps as an efficient monitoring method. Through quantitative and qualitative analysis of entomological communities collected in the vicinity of experimental fields treated with herbicides and insecticides, the research aims to: identify the main dominant taxonomic orders in the targeted agricultural area; establish possible correlations between the activity of beneficial insects and plant protection practices; contribute to the development of sustainable strategies for managing functional biodiversity in agricultural ecosystems.

Materials and methods:

The monitoring of insect entomofauna was carried out in the period April–July 2020–2021. The suction trap produced by MEKU-Erich Pollähne GmbH was used to collect insects from the experimental field area. This trap allows the collection of arthropods at a height of between 1.65 and 2 meters above the ground, with a suction power of approximately 32 m^3/min. The flow velocity reaches values between 2 and 3 m/s, at a distance of 250 mm from the suction center.

A REITZ fan is installed inside the trap, mounted on four vibration dampers. The attracted insects were collected using a funnel, at the end of which were 500 ml plastic containers, which were changed weekly. Sample collection was controlled using a programmable timer. The device operated during the week between 9:00 a.m. and 7:00 p.m. After each collection session, the system automatically returned to the rest (closed) position, thus preventing particles from entering the containers during breaks in operation.

All samples collected from the study area were identified according to reference works. The identification process was further supported by entomologists from the Landwirtschaftskammer NRW–Köln, who have significant expertise in insect taxonomy.

The diversity of insect species in the study area was analyzed by calculating recognized ecological indices for assessing biodiversity, such as the Margalef index, the Shannon–Wiener Diversity index and the Simpson index. These parameters were used to explain the structure of the entomological community, taking into account the variety, evenness and dominance of the identified species within the analyzed agroecosystem.

Results and conclusions:

In the two years of capturing insects and spiders using the suction device, 101,232 specimens were collected, of which 50,573 in 2020 and 50,659 in 2021.

In the two years, the total number of captures was relatively constant, confirming the stability of the populations within the investigated ecosystem.

The species collected systematically belong to 17 families from 9 orders.

From the order Diptera, 49,095 specimens were captured, representing 48.5% of the captures of the two years.

From the order Thysanoptera, 24,595 specimens were captured, representing 24.3% of the total captures.

From the order Coleoptera, 8,850 specimens were captured, representing 8.7% of the total captures.

The spiders captured numbered 1098 specimens, representing 1.1% of the total catches.

From the orders Hemiptera, Lepidoptera. Hemiptera, Neuroptera and Psocoptera, 117594 specimens were captured, representing 17.3% of the total catches.

Among the zoophagous species, specimens from the families Staphylinidae, Coccinellidae and Cantharidae (order Coleotera), from the families Ichneumonidae and Proctotrupidae (order Hemiptera) and the family Chrysopidae (order Neuroptera) were captured.

Biodiversity indices highlighted trends in community restructuring through variations in abundance, richness and dominance, with major functional implications on ecosystem stability and ecosystem services provided by useful entomofauna.

RECOMMENDATION

1. For the detection and monitoring of the numerical density of the Drosophila suzukii population, it is recommended to use food baits produced at the "RALUCA RIPAN" Chemistry Research Institute, UBB Cluj-Napoca, and for Thrips setosus, it is recommended to place fluorescent yellow sticky panels or the Berlese–Tullgren method.

2. These methods are recommended to be applied in organic crops to combat pests by mass capture of adults.

3. In biodiversity research studies, the use of the suction device allows for a proper assessment of the structure of arthropod species within the entomofauna.

INNOVATIVE CONTRIBUTIONS OF THE THESIS

The *Drosophila suzukii* species was monitored in two areas of the North Rhine-Westphalia region, Germany (Köln and Meckenheim).

For monitoring, three variants of food baits produced at the "RALUCA RIPAN" Chemistry Research Institute, UBB Cluj-Napoca, were used, and the effectiveness of each bait in capturing adults of Drosophila suzukii was established in order to be recommended in the practical activity of farmers.

In the catches of *Drosophila suzukii,* their sex structure was determined.

The *Thrips setosus* species (a recently reported species in the investigated area) was monitored on three varieties of *Hydrangea macrophylla* by using colored sticky traps and their attractiveness performance was established.

For *Thrips setosus,* the Berlese–Tullgren method was used for the first time.

In the catches of *Thrips setosus,* the mobile stages of development (adults and larvae) were determined, and in adults, their sex structure was established.

The suction device was used to assess the biodiversity of arthropods in the North Rhine-Westphalia (NRW) region of Köln.

It established the structure of the collected entomofauna and, by calculating biodiversity indices, assessed the evolutionary trend of species in 9 orders.

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