PhD THESIS

Protection management of fruit bush plantations in context of current climate changes in Transylvania

(SUMMARY OF Ph.D. THESIS)

PhD student Ionuț-Bogdan Hulujan

Scientific coordinator Prof. univ. dr. Teodora Florian



Introduction

In order to effectively control harmful species, it is necessary to accurately identify them first, taking into account their morphological and biological characteristics, and then choose and implement appropriate control measures. Protecting crops from harmful species can only be achieved through the rational application of a comprehensive set of measures, developed based on the latest advances in science and agricultural practices.

Due to current legislation and the environmental impacts (pollution, residue accumulation, disruption of ecological balance), the methods used must be ecologically friendly, efficient, and applicable on a large scale. In the case of pests in fruit bush crops, the focus is on preventing attacks and subsequently controlling them.

1. Structure of the doctoral thesis

The doctoral thesis includes a total of 150 pages and was structured in two parts:

- current state of knowledge part I (36 pages)
- personal contribution part II (114 pages)

The second part of the thesis includes 114 pages and is divided into 6 chapters, which present the objectives, the description of the experimental area, materials and methods used, results obtained and discussions, conclusions and recommendations based on the results, as well as the originality and innovative contributions of the research. The thesis includes a total of 27 tables, 48 figures and 218 bibliographic references.

2. Objectives

For the elaboration of the doctoral thesis, the following objectives were proposed:

2.1. Collection of fauna from fruit bush plantations using Barber traps

To achieve this objective, starting from 2021, 6 Barber traps were installed in 5 locations: the fruit shrub collection at UASMV Cluj-Napoca, the sea buckthorn plantation in Sâncrai, Alba County, the black currant plantation in Recea-Cristur, Cluj County, the blackberry plantation in Buciumi, Maramureş County, and the raspberry plantation in Buciumi, Maramureş County.

After collection, the biological material was tracked: identification and systematic classification of the biological material; analysis of the trophic base of the identified species and calculation of ecological indices for the captured beetles in the 5 locations.

2.2. Detection and monitoring of *Drosophila suzukii* Matsumura species

For this objective, the following aspects were studied in the blackberry collection at UASMV Cluj-Napoca:

- capture and identification of *Drosophila suzukii* Matsumura species;
- variation in the susceptibility of blackberry varieties to infestation by *Drosophila suzukii*;
- testing of food baits in the monitoring of *Drosophila suzukii* species.

These food baits were analyzed in terms of number of captures, the gender structure of the collected biological material, and their selectivity.

2.3. Detection and monitoring of Phyllopertha horticola Linné species

For this objective, in the blueberry plantation in Mărişel, Cluj County, the identification of *Phyllopertha horticola* Linné species and the determination of the frequency of attacked plants were carried out.

2.4. Monitoring and control of some pests in black currant plantations

For this objective, in the black currant plantation in Recea-Cristur, Cluj County, the following aspects were studied: testing of semiochemical products in the monitoring of *Oxythyrea funesta* Poda and *Tropinota hirta* Poda; determination of the frequency and intensity of damage caused by defoliators and control of defoliators using products approved for organic production.

3. Material and methods

3.1. Collection of fauna from fruit bush plantations using Barber traps

Over the course of two experimental years, 6 Barber traps were placed and 12 collections were made at two-week intervals from May to the end of October.

The collected specimens were identified and classified based on their external morphology and then grouped according to their trophic base, into phytophagous species and zoophagous or coprophagous species.

Ecological indicators such as abundance, dominance, constancy, and the ecological significance index were analyzed for the collected biologic material.

3.2. Detection and monitoring of *Drosophila suzukii* Matsumura species

The studies on the variation in susceptibility of blackberry cultivars to infestation by *Drosophila suzukii* Matsumura were conducted in the blackberry collection at the UASMV Cluj-Napoca. The studied blackberry cultivars were Thornless, Loch Ness, Thornfree, Thornless Evergreen and Navaho.

One hundred fruits were randomly harvested in three repetitions at the onset of ripening, and the frequency of attacked fruits and the number of larvae per attacked fruit were evaluated in Entomology laboratory of the UASMV Cluj-Napoca.

For monitoring *Drosophila suzukii* Matsumura, three variants of synthetic food baits synthesized at the "Raluca Ripan" Institute for Research in Chemistry, UBB Cluj-Napoca, were tested. The tested variants were:

- \triangleright V₁ apple vinegar (75%), Merlot wine (24%), brown sugar and liquid apple soap (1%);
- \triangleright V₂ apple vinegar (99%), brown sugar and liquid apple soap (1%);
- ➤ V₃ apple vinegar (50%), Merlot wine (49%), brown sugar and liquid apple soap (1%). The traps were set up annually in the beginning of March and weekly collected.

3.3. Detection and monitoring of Phyllopertha horticola Linné species

Observations were carried out in the blueberry plantation, Mărișel, Cluj County, in the year 2021 on July 7, two varieties being analyzed.

For monitoring the garden chafer, *Phyllopertha horticola* Linné, in each plot, three rows with intervals of 20 plants per row were randomly selected. Each plant was individually analyzed, and the number of adults per plant was recorded.

3.4. Monitoring and control of some pests in black currant plantations

For monitoring the species *Oxythyrea funesta* Poda and *Tropinota hirta* Poda, in the period 2020-2022, adults were collected using blue traps with water and environmentally friendly products placed in blue containers. The studies were conducted in an organic blackcurrant plantation in Recea-Cristur, Cluj County. Three repetitions of each variant were placed annually. The traps were set up in the last decade of April, and the captures were monitored until the end of May. Statistic analysis of the obtained results was performed using the Anova trifactorial software.

The experimental variants were:

- \triangleright V₁ blue traps with water (the control);
- ➤ V₂ (E)-anethole & (E)-cinnamyl alcohol;
- V_3 (±)-lavandulol & 2-phenylethanol.

For determine the intensity of the attack produced by defoliators in the blackcurrant plantation in Recea-Cristur township, Cluj County, on 8.09.2021 and 7.09.2022 respectively, 100 plants were randomly chosen from each variety or variant.

For the control of defoliators, approved products for organic production were used. In order to determine the effectiveness of the treatments, eight treatments were applied annually, and the degree of infestation was compared between the treated and untreated variants.

4. Results and discussions

4.1. Results regarding fauna collection from fruit bush plantations using Barber traps

In the fruit bush collection at UASMV Cluj-Napoca, 9704 specimens were collected in 2021, and 11727 specimens were collected in 2022, showing an increase of approximately 21% in the number of captures. In 2021, out of the total captures, 89,1% belonged to the Phylum Arthropoda, and 10,9% belonged to the Phylum Mollusca. In 2022, arthropods represented 99,1% of the captures, while specimens from the Phylum Mollusca represented 0,9%.

The phytophagous species accounted for 48,3% of the total captures in 2021, while the zoophagous or coprophagous species accounted for 51,7%. In 2022, phytophagous species represented 35,5% of the total captures, while zoophagous species accounted for 64,5%.

The specimens collected from the Phylum Arthropoda belong to four subphyla. In 2021, 5858 specimens were collected from the Subphylum Hexapoda, representing 67,74% of the total captures. From the Subphylum Chelicerata, 1325 specimens were collected, accounting for 15,32%. The Subphylum Crustacea accounted for 1265 specimens, representing 14,63% of the total captures, and the Subphylum Myriapoda accounted for 200 specimens, representing 2,31%. Among the 11623 arthropods

collected in 2022, the rate of hexapods was 70%, the rate of chelicerates was 18,08%, the rate of crustaceans was 9,93%, and the rate of myriapods was 1,61%.

Within the Class Insecta, 4923 specimens were collected in 2021, and 7375 specimens were collected in 2022, belonging to eight orders. This is the only location where diptera were identified, as there were 186 specimens of *Drosophila suzukii* Matsumura in 2021 and 64 specimens in 2022, an invasive species, reported and identified for the first time in the collection of fruit bushes of UASMV Cluj-Napoca in 2020 with the help of the trap with food bait. Of the total insects, in the first year of monitoring, 48,1% were phytophagous, and 51,9% were zoophagous or coprophagous. A total of 39,4% of the insects collected in 2022 were phytophagous, while 60,6% were zoophagous or coprophagous.

The Order Coleoptera is the most prevalent within the Class Insecta. 3853 specimens were collected in 2021, accounting for 78,27% of the total captured insects. These come from 22 families and 128 species. 4608 specimens, or 62,48%, were collected in 2022. Coleopterans this year comprise 179 species from 26 families. Among the families containing species of beneficial insects, the most important are the Family Carabidae, with 1783 specimens of 45 species in 2021 and 3105 specimens of 50 species in 2022. Notable species include *Abax parallelus* Duftschmid, *Harpalus distinguendus* Duftschmidt, *Calathus fuscipes* Goeze, *Pseudoophonus rufipes* DeGeer, *Brachinus crepitans* Linné, *Nebria brevicollis* Fabricius, and *Carabus violaceus* Linné, as well as the Family Staphylinidae with *Drusilla canaliculata* Fabricius.

Among the families with phytophagous species, the Nitidulidae family with *Stelidota geminata* Say, the Chrysomelidae Family, and the Curculionidae Family stand out.

In the sea buckthorn plantation located in Sâncrai, Alba County, a total of 10997 specimens were collected in 2021, and 24754 specimens were collected in 2022, representing an increase of 2,25 times.

Based on trophic categories, in 2021, phytophagous species accounted for 61,2% of the total captures, while zoophagous or coprophagous species accounted for 38,8%. In 2022, phytophagous species represented 74,8% of the total captures, while zoophagous species accounted for 25,2%.

The species from the Phylum Arthropoda belong to four subphyla: Crustacea ranged from 51,09% to 69,74%, with the highest value among the five locations; Hexapoda ranged from 17,8% to 25,52%; Chelicerata ranged from 11,53% to 23,01%; and Myriapoda ranged from 0,38% to 0,93%.

In both years, the majority of collected insects belonged to the Order Coleoptera. In 2021, 1379 specimens were collected, representing 77,17% of the total captured insects, and in 2022, 2250 specimens were collected, representing 69,19%. In 2021, these beetles belonged to 112 species from 23 families, while in the following year, there were 133 species from 23 families. The families best represented in 2021, in terms of the number of collected specimens and/or species, were Lampyridae (with the notable

zoophagous species *Phosphaenus hemipterus* Geoffroy), Staphylinidae with 21 species (including the zoophagous species *Drusilla canaliculata* Fabricius), Carabidae with 29 species (including the zoophagous species *Brachinus crepitans* Linné) and among the families with phytophagous species: Chrysomelidae with 15 species, Nitidulidae with 2 species attacking floral organs, and Curculionidae with 11 species.

In 2022, the majority of specimens collected among zoophagous species were from the Family Carabidae with 39 species (including the species *Brachinus crepitans* Linné), Staphylinidae with 25 species (including the species *Drusilla canaliculata* Fabricius, *Ocypus brunnipes* Fabricius, and *Staphylinus caesareus* Cederhjelm), and Lampyridae (including the zoophagous species *Phosphaenus hemipterus* Geoffroy). Among the phytophagous species, the highest number of collected specimens were from the Family Chrysomelidae with 16 species.

In the blackcurrant plantation located in Recea-Cristur, Cluj County, a total of 8595 specimens were collected in 2021 and 27274 specimens were collected in 2022. This location had the highest increase in captures in 2022, with a growth of 3,17 times compared to the previous year.

In 2021, out of the 7400 collected arthropod specimens, hexapods represented 66,46%, chelicerates 29,64%, myriapods 2,86%, and crustaceans 1,04%. In 2022, hexapods represented 80,37%, 17,33% were chelicerates, 1,4% were crustaceans, and 0,9% were myriapods.

In 2021, phytophagous species accounted for 26,8% of the captures, while species from the beneficial fauna represented 73,2%. In 2022, phytophagous species accounted for 18,7% of the captures, while species from the beneficial fauna represented 81,3%. This location consistently showed the lowest participation of phytophagous species in the insect structure each year.

The majority of collected insects in 2021 belonged to the Order Coleoptera, representing 86,96% of the total captures. These belonged to 151 species from 23 families. In the second year, the order Coleoptera represented 88,19% of the total captured insects, belonging to 183 species from 30 families.

The families best represented in terms of the number of collected specimens and/or species were the Family Carabidae with 35 species in 2021 and 57 species in 2022 (including *Abax parallelus* Duftschmid, *Calathus fuscipes* Goeze, *Brachinus crepitans* Linné, and *Pseudophonus rufipes* DeGeer) and the Family Staphylinidae with 30 species in 2021 and 23 species in 2022 (including *Aleochara haematodes* Schaum, *Quedius molochinus* Gravenhorst and *Staphylinus caesareus* Cederhjelm). The species from these families are zoophagous.

In the blackberry plantation in Buciumi (Maramureş County), 3651 specimens were collected in 2021, and 3438 specimens were collected in 2022. This is the only location where the number of captures decreased in the second year of the study.

In 2021, phytophagous species accounted for 37,7% of the total captures, while zoophagous species accounted for 62,3%. In 2022, phytophagous species represented

22,5% of the total captures, while zoophagous or coprophagous species accounted for 77.5%.

In 2021, out of the total collected specimens, 74,01% were from Hexapoda, 23,41% were from Chelicerata, 1,54% were from Myriapoda, and 1,04% were from Crustacea. In 2022, 61,89% were from Hexapoda, 35,17% were from Chelicerata, 2,08% were from Myriapoda, and 0,86% were from Crustacea.

Among insects in the first year of monitoring, the majority of collected specimens belonged to the Order Coleoptera, representing 59,82%. These belonged to 117 species from 23 families. The families with zoophagous species that were best represented in terms of the number of collected specimens and/or species were the Family Carabidae with 30 species (including *Calathus fuscipes* Goeze, *Pseudoophonus calceatus* Duftschmid and *Abax parallelus* Duftschmid) and the Family Staphylinidae with 25 species (including the zoophagous species *Drusilla canaliculata* Fabricius). Among the families with phytophagous species the Family Curculionidae had 17 species (notably *Xyleborus germanus* Blandford) and the Family Nitidulidae (*Stelidota geminata* Say).

In 2022, the collected specimens from the Order Coleoptera represented 69,71% of the total Insecta class. These belonged to 115 species from 20 families. The families with zoophagous species that were best represented were the Family Carabidae with 38 species (including *Calathus fuscipes* Goeze, *Abax parallelus* Duftschmid and *Pseudoophonus rufipes* DeGeer) and the Family Staphylinidae with 18 species (including the species *Drusilla canaliculata* Fabricius).

In the raspberry plantation in Buciumi, Maramureş County, 5633 specimens were collected in 2021, and in 2022, the number of collected specimens increased by over 1.3 times, reaching 7473 specimens.

Phytophagous species accounted for 58,2% in 2021 and 48,1% in 2022, while zoophagous or coprophagous species accounted for 41,8% in the first year and 51,9% in 2022, out of the total collected specimens.

In 2021, the collected specimens from the Subphylum Hexapoda were 4150, representing 80,76% of all arthropods; 9,71% were from Chelicerata, 8,74% were from Crustacea and 0,79% were from Myriapoda. In 2022, there was a significant decrease in the collected specimens from the Subphylum Hexapoda, with their representation in the arthropod structure dropping to only 49,67%. However, the percentage of specimens from the other subphyla increased: 29,23% were from Crustacea, 19,97% were from Chelicerata and 1,13% were from Myriapoda.

In 2021, out of the order Coleoptera, 3280 specimens were collected, representing 83,82% of the total captured insects. They belonged to 91 species from 19 families. In 2022, beetles represented 65,27% of the total captured insects. They still belonged to 91 species from 18 families.

Among the families with zoophagous species, the Family Carabidae was well represented with 1652 specimens from 23 species (including the zoophagous species *Abax parallelus* Duftschmid, *Nebria brevicollis* Fabricius and *Pseudophonus rufipes*

DeGeer) and the Family Staphylinidae with 113 specimens from 14 species (with the highest number of specimens being *Drusilla canaliculata* Fabricius).

4.2. Results regarding the detection and monitoring of the *Drosophila* suzukii Matsumura species

In 2020, the highest frequency of attacked fruits was reported in the Thornfree variety, reaching 89%. The Thornless Evergreen variety had an average attack frequency of 82%. The lowest attack frequency was observed in the Loch Ness variety, with an average of 71%, followed by the Navaho variety with an average of 72%. In the blackberry plantation, the average frequency of attacked fruits this year is 78,2%.

In 2021, the frequency of attacked fruits was significantly lower compared to 2020 for all varieties. Across the entire blackberry collection, the attack frequency in that year was 59,6%, a decrease of 18,6% compared to the previous year. Among the varieties, the average frequency of attacked fruits ranged from 49% in the Thornless Evergreen variety to 66% in the Thornless variety.

In 2020, the maximum number of larvae reported in a single fruit ranged from 17 larvae in the Thornless variety to 48 larvae in the Loch Ness variety. In 2021, the range was from 11 larvae in the Thornfree variety to 21 larvae in the Navaho variety.

In 2020, the average number of larvae per fruit ranged from 4,4 larvae in the Thornless variety to 9,4 larvae in the Loch Ness variety. Across the entire blackberry collection, this parameter was 6,4 larvae per attacked fruit. In 2021, the average number of larvae per fruit ranged from 2,8 larvae in the Thornfree variety to 4,2 larvae in the Navaho variety. Across the entire blackberry collection, this parameter was 3,4 larvae per attacked fruit.

Among the tested eco-friendly variants for *Drosophila suzukii* Matsumura, Variant V_1 - apple vinegar (75%), Merlot wine (24%), brown sugar and liquid apple soap (1%), achieved the highest captures in both years. Thus, in 2021, the captures represented 48,4%, and in 2022, it accounted for 44,1% of the total annual captures.

Variant V_2 - apple vinegar (99%), brown sugar and liquid apple soap (1%), had the lowest captures. The percentage of captures was 24,4% in 2021 and 20,3% in 2022 of the total annual captures.

Therefore, in 2021, males represented between 63,5% and 67,2% of the total captures, and in 2022, they accounted for between 58,8% and 72,4% of the total annual captures, with an average of 63,3%.

4.3. Results regarding the detection and monitoring of the *Phyllopertha horticola* Linné species

For the Elliot variety, the proportion of plants on which adults of *Phyllopertha horticola* Linné were reported ranged from 50% to 60%, with an average of 55%. The maximum number of adults per plant ranged from 6 to 9 specimens.

In the Bluecrop variety, the proportion of plants with adults of the monitored species ranged from 10% to 45%, with an average of 25% per variety. The maximum number of adults per plant ranged from 2 to 4 specimens.

4.4. Results regarding the monitoring and control of some pests in black currant plantations

In the variant V_2 – (E)-anethole & (E)-cinnamyl alcohol, over the course of three years (2020-2022), a total of 318 specimens were captured, out of which 282 were *Tropinota hirta* Poda (representing 88,7% of the total captures) and 36 were *Oxythyrea funesta* Poda. The highest number of captures of *Tropinota hirta* Poda was obtained in 2021, with 149 adults, accounting for 52,8% of the total captures during the testing period.

In the variant V_3 – (±)-lavandulol & 2-phenylethanol, over the course of three years (2020-2022), a total of 218 specimens were captured, out of which 139 were *Oxythyrea funesta* Poda (representing 63,8% of the total captures) and 79 were *Tropinota hirta* Poda (representing 26,2% of the total captures).

By applying chemical treatments against defoliators, in 2021, the level of attack was reduced in all varieties. The effectiveness of the applied treatments ranged from 46,4% (for the Tisel variety) to 56,5% (for the Ruben variety). In 2022, the effectiveness of the chemical treatments ranged from 67,1% (for the Ruben variety) to 77,9% (for the Tiben variety).

5. Conclusions and recommendations

During the two years of invertebrate monitoring, a total of 113246 specimens were collected from the study locations, of which: 35869 specimens from the blackcurrant plantation in Recea Cristur, Cluj County; 35751 specimens from the sea buckthorn plantation in Sâncrai, Alba County; 21431 specimens from the fruit shrub collection at USAMV Cluj-Napoca; 13106 specimens from the raspberry plantation in Buciumi, Maramureş County and 7089 specimens from the blackberry plantation in Buciumi, Maramures County.

The structure and numerical density of invertebrate populations from the Phylum Arthropoda and the Phylum Mollusca are influenced by the composition of the local vegetation cover, the cultivation technology used in the plantation, and the local soil and climate conditions.

The percentage of phytophagous species in the fauna structure of a crop is a complex process influenced by the applied technology, but it is also affected by the presence of zoophagous insect species, which play an essential role.

Among the beneficial fauna, in addition to the species from the subphylum Chelicerata, species of beetles were notable, such as *Nebria brevicollis* Fabricius, *Calathus fuscipes* Goeze, *Pseudoophonus rufipes* DeGeer, *Brachinus crepitans* Linné, *Poecilus cupreus* Linnaeus, *Harpalus affinis* Schrank, (all from the Family Carabidae), *Drusilla canaliculata* Fabricius (Staphylinidae) and *Phosphaenus hemipterus* Goeze (Lampyridae).

In each year and at each location, the majority of captures were from the class Insecta, with the Order Coleoptera being the most prominent. The most representative families of beetles in terms of species number are Carabidae and Staphylinidae. Combined across all five locations and the two years of the study, a total of 42973 beetle specimens were captured, representing 525 species.

In terms of the collection, the average number of larvae per attacked fruit of *Drosophila suzukii* Matsumura was 6,4 larvae in 2020 and 3,4 larvae in 2021.

The maximum number of larvae observed in a single attacked fruit in 2020 was 48 larvae in the Loch Ness variety, while in 2021, it was 21 larvae in the Navaho variety.

For the species *Tropinota hirta* Poda, the bait (E)-anethole & (E)-cinnamyl alcohol was effective, accounting for 88,7% of the total captures during the three-year experimental period. For the species *Oxythyrea funesta* Poda, the bait (±)-lavandulol & 2-phenylethanol was effective, representing 63,8% of the total captures during the study period.

The effectiveness of chemical treatments to control defoliators in blackcurrant plants in 2021 was 46,4% for the Tisel variety, 54,1% for the Tiben variety, and 56,5% for the Ruben variety. In 2022, the effectiveness was 66,8% for the Tisel variety, 67,1% for the Ruben variety, and 77,9% for the Tiben variety.

Recommendations

The monitoring of Barber traps is an accessible method for all farmers to assess the fauna in fruit shrub crops.

Monitoring the dynamics of pest population density is essential for determining the most suitable control strategies, especially in organic farming.

The use of food baits and eco-friendly baits yields excellent results in monitoring species such as *Drosophila suzukii* Matsumura, *Tropinota hirta* Poda, and *Oxythyrea funesta* Poda.

6. Originality and innovative contributions of the thesis

The monitoring of the invertebrate fauna was carried out for the first time in the five locations studied (Cluj-Napoca, Cluj County – collection of fruit trees (UASMV Cluj-Napoca); Sâncrai, Alba County – sea buckthorn plantation; Recea-Cristur, Cluj County – blackcurrant plantation; Buciumi, Maramureş County – blackberry plantation and Buciumi, Maramureş County – raspberry plantation).

The structure of the fauna of zoophagous arthropods from the plantations of fruit trees cultivated in the monitored areas was studied.

In the investigated area the phytophagous species *Stelidota geminata* Say (Coleoptera, Nitidulidae), *Xyleborus germanus* Blandford (Coleoptera, Curculionidae) and *Drosophila suzukii* Matsumura (Diptera, Drosophilidae) were reported for the first time.

The effectiveness of some food baits synthesized at the "Raluca Ripan" Institute for Research in Chemistry, UBB Cluj-Napoca, was tested for the first time for monitoring the species *Drosophila suzukii* Matsumura.