Ph.D. thesis

Predatory mites in Hungarian vineyards and apple orchards

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I. INTRODUCTION AND RESEARCH OBJECTIVES

The biological control of pest mites is principally based on the use of phytoseiid species in the case of plantations, especially vineyards and apple orchards in Hungary. The knowledge of species composition of predatory mites, their occurrence within a plantation and also their migration characteristics can give the opportunity for exploiting these beneficial arthropods in pest control. Several scientific papers report on the effective application of predatory mites in biological control also in the case of orchards. However, the climatic conditions and consequently the species composition of predatory mites in wine and apple growing regions of Hungary partly differ from those of other countries. The significance of beneficial mite populations and also their dynamics are determined by both biotic and abiotic factors, so the study of these effects is a must before adapting any biological control methods developed in other countries of different climatic regions. Hence the occurrence and relative dominance of phytoseiid mite species need to be investigated in Hungarian orchards. In biological pest control, experiments with species potentially better adapted to the local conditions should also be carried out. For a first step in this field of research, I planned to make detailed faunistic studies in Hungarian wine growing regions. Not only the species living on cultivated plants but also those occurring on plants between rows or in the surrounding vegetation, which latter is known to have a major effect on the natural dispersal of predatory mites, were aimed to be investigated. As a further objective, the identification of phytoseiid species collected in different plantations was targeted. This study was based on and results were compared with data found in the relevant Hungarian scientific literature and current taxonomic information.

For the development of biological control methods by the application of predatory mites, I aimed to reveal details of spatial and temporal changes of mite populations within the plantations. In the case of apple orchards, mite communities consisting of certain species of economic importance are present in high numbers on the foliage during the vegetation period, but they similarly occur frequently in the ground litter during the dormancy period. The effect of populations living in the latter biotope on the forming of mite communities of the foliage is only partly clarified. In this respect, I aimed to reveal the interrelationship between populations of spider and predatory mites living in the ground litter and on the foliage, and I also tried to determine its significance in plant protection. The main objective of these studies was to find out whether there are any mechanical methods with the help of which the migration of *Tetranychus urticae* Koch can be hindered in a given orchard. These examinations made it possible to get information on the places where phytoseiid species can overwinter and also on the way how they get there.

Control of pest mites within a plantation is possible by saving their natural enemies applying environmentally friendly plant protection technologies or even by releasing predatory mites. Many examples of successful release of beneficial mites are known from Europe. The release of *Typhlodromus pyri* Scheuten by using textile strips has become quite common in Hungarian wine growing regions, too. In my work, I evaluated the bases of this technology and the effectiveness of the mentioned mite species when released in apple orchards. The possibilities of introducing another predatory mite species, *Amblyseius andersoni* Chant was also investigated based on my newly developed method. The aim of this experiment was to demonstrate that *A. andersoni*, which overwinters in the ground litter, may be introduced to orchards by carrying dead fallen leaves from one plantation to another. According to my hypothesis, those species which overwinter in the ground litter and move to the canopy during the vegetation period and finally move back to the soil level in autumn may be introduced to orchards with the help of fallen leaves.

II. MATERIALS AND METHODS

Studies in vineyards

My studies to reveal the predatory mite fauna of Hungarian vineyards by collecting leaf samples was carried out in the wine growing regions of Ászár-Neszmély, Eger, Somló, Sopron, Tokaj-hegyalja and Villány, between 2004 and 2009. With the exception of Eger, there were not any faunistical data found in literature representing the region. Each studied vineyard was characterised by cultivars and growing methods typical of the given wine growing region. In the majority of the vineyards, conventional or integrated pest management was applied, but a small amount of samples originated from abandoned plantations or vineyards where there was biological production.

Leaf samples were taken in the growing season, and the lower surface of leaves was examined with a stereo microscope. In the dormancy period, woody parts of grapevine as well as fallen leaves and dead weeds from the ground litter were collected. Mite specimens were extracted with a Berlese-Tullgren funnel under 24-hour lighting.

In some vineyards of the Tokaj-hegyalja and Eger wine regions, 30-30 leaves per sampling date and per a certain plant species deriving from the vineyards or their surrounding vegetation were also examined with a stereo microscope in the laboratory to collect phytoseiid species.

Experiments in apple orchards

a) Study on the populations living in the ground litter

Research on the predatory mite species which overwinter in the ground litter was carried out in an apple orchard at Jakabszállás. Dead fallen leaf samples were taken both from and between rows within the plantation. In both cases, there were 12 replicates each consisting of 15 leaves. In the laboratory, the Berlese-Tullgren funnel was used to collect predatory mite specimens and compare the species composition of the two different places.

My studies on hindering spider mite populations, which overwinter in the ground litter and reproduce on weeds, to move to the foliage were conducted between 2007 and 2009. So as to stop crawling of mites from the soil level to the canopy, in certain plots, the trunk of each tree was treated with non-drying adhesive at a height of 40 cm at the end of the dormancy period. Trees in control plots were not treated with the material. 100 leaves were collected biweekly from both the treated and the control plots to evaluate the efficiency of the treatment. In 2009, the experiment was repeated at Soroksár. In this case, I collected 25-25 leaves in 4 replicates per both the treated and the control plots. In the laboratory, the lower side of the leaves was examined with a stereo microscope. Motile stages of phytoseiid species were picked carefully from the leaves. I also made notes of the location of phytoseiid eggs found on the leaves. I marked those eggs which were not laid on the lower epidermis directly but onto trichomes or the net of spider mites. To count apple rust mites, not the whole leaf surface but a leaf size of 28 mm^2 (40x) chosen randomly was taken.

b) The release of phytoseiid species

My studies were carried out in a three-year-old apple orchard of cv. Golden Reinders in the Experimental Station of the Corvinus University of Budapest. Environmentally friendly plant protection technology was applied to control pests in both years of this study (2008 and 2009).

Amblyseius andersoni was introduced into the plantation by ground litter, which can be regarded as a new method. The ground litter collected from the rows of the ‘donor-orchard’ was placed in the test orchard on the same day. Two litres of ground litter (approx. 25 overwintering *A. andersoni* females) clamped with a small mesh-size net were attached to the tree trunks.

The OP-resistant ’Mikulov’ strain of *Typhlodromus pyri* originating from the Czech Republic was also introduced into the orchard in the dormancy period before starting the studies. For this purpose, textile strips (distributed by Biocont Magyarország Kft.) were used.

Leaf samples were taken every second week during the vegetation period. In 2008 and 2009, 27-27 leaves in 5 replicates and 25-25 leaves in 4 replicates, respectively, per both the treated and the control plots were collected. For the quantitative analysis of mite species, I used the method described in the a) subsection.

Identification of mite species

All the predatory mite species belonging to the order Mesostigmata and indicated in my Ph.D. thesis have basically been identified by the taxonomic works of Karg's (1971, 1991, 1993). As according to other specialists the taxonomic status of some species differs from that given by Karg, the different name in the case of certain species is also shown and is based on the Fauna Europaea database. In many cases, the works of other acarologists had to be used as well for the identification, e.g. Westerboer (1963), Chant and McMurtry (1994, 2003a, 2003b), Miedema (1987), Edland and Evans (1998), Ferragut and Escudero (1997) and Chant and Yoshida-Shaul (1982, 1983, 1984, 1987, 1989). For the identification of *Anthoseius* species, further papers and original species descriptions have also been used. The taxonomic status of tetranychid species has been determined with the help of Bozai's (1970) key, but the study of Baker and Tuttle's (1994) was also taken into consideration. Specimens of the identified species have been deposited in a newly created Acari Collection of the Department of Entomology, Corvinus University of Budapest. Some microscopic images of the most important diagnostic characters of the identified species are shown in the Appendix chapter of my thesis.

Statistical analysis

Statistical analysis was carried out to compare the number of phytoseiid mites and other taxa by treatments and sampling dates. As the data often did not conform to a normal distribution, the abundances were compared by the Mann-Whitney U test. In other cases, ANOVA had to be used. The statistical analyses were carried out by using the Past software package. Detailed results are shown in the Appendix chapter of my thesis.

III. RESULTS

Faunistical studies

a) The predatory mite fauna of the hilly wine regions of Hungary

As a result of my surveys in vineyards, 20 phytoseiid species have been identified. 2 out of the 20 species were only collected from the ground litter, whereas the remaining 18 species occurred on the woody parts and leaves of grapevine as a host plant. 11 out of the 18 species occurred exclusively on grapevines, compared to the remaining 7, which were detected both on the grapevines and in the ground litter. On top of the 20 phytoseiid species mentioned, 7 other mesostigmatid species were determined. 3 out of these (*Ameroseius pavidus* (Koch), *Leioseius bicolor* (Berlese) and *Arctoseius cetratus* (Sellnick)) were collected from the woody parts, and the other 4 (*Proctolaelaps pygmaeus* (Müller), *Lasioseius fimetorum* Karg, *Lasioseius youcefi* Athias-Henriot and *Asca bicornis* (Canestrini et Fanzago)) from the ground litter.

15 out of the 18 phytoseiid species collected from grapevine have been known in Hungary before. However, 8 species (*Blattisocius tarsalis*, *Amblyseius agrestis*, *A. cucumeris*, *A. reductus*, *A. lutezhicus*, *P. triporus*, *Anthoseius hungaricus*, *A. involutus*) have been collected from grapevine for the first time, and another 3 species (*Amblyseius neobernhardi*, *Anthoseius richteri* and *A. rivulus*) have been included in the Hungarian fauna list for

the first time within the framework of my studies. All the mesostigmatid species are new on the list of species collected from grapevine. Moreover, *Ameroseius pavidus* is new to the Hungarian fauna. Data on the distribution of these species in Hungary are shown in tables of my thesis.

Typhlodromus pyri was the most common; it occurred in 70 out of the 82 vineyards (85%) examined across the country. It was the most common species in each wine region surveyed. *Amblyseius andersoni* was the subdominant species (17%) and the third most prevalent species was *Euseius finlandicus* (15%).

A diverse Phytoseiidae assemblage was found in the samples collected from other plant species in the vineyards and the surrounding vegetation. 11 out of the 18 identified predatory mite species occurred on grapevine, too. 2 species have been found in the Hungarian fauna for the first time; *Blattisocius keegani* Fox, and *Typhlodromus bichaetae* Karg.

b) Miscellaneous results of faunistical studies

Although it was not a main objective of my work, the distribution of phytoseiids and other mesostigmatid species was investigated also in other fields of horticulture. 26 predatory mite species of the order Mesostigmata have been identified from apple orchards. 3 species (*Anthoseius occiduus*, *Anthoseius pirianykae*, *Veigaia planicola*) out of them have been recorded in Hungary for the first time.

The role of ground litter of apple orchards in the population dynamics of mite communities

a) The presence of phytoseiids in apple orchards during the dormancy period

Significantly more phytoseiid specimens were found on the fallen leaves in rows, especially around the tree trunks, than between rows. On the other hand, by examining autumn leaves, I established that the number of *A. andersoni* and *E. finlandicus* on yellowing leaves hanging on the trees was significantly lower, than on green leaves, thus these species reached their places of overwintering by something rather than leaves.

b) Interrelationship between mite populations living in the ground litter and on the foliage in apple orchards

The two spotted spider mite was found to be the dominant pest mite species in both experimental orchards and also in both years of the study. The treatment of tree trunks with non-drying adhesive resulted in a significant difference as far as the abundance of the species is concerned. This difference could be observed in one month in 2007, but in the whole vegetation period in 2009. In the latter case, the number of TSSM on treated trees was lower than on the control trees as early as May. A similar effect of this mechanical treatment could be observed in the case of predatory mites as well.

The release of phytoseiids in apple orchards

a) The new method of release of *A. andersoni*

In these studies, the primary prey of predatory mite species was the apple rust mite in 2008, while it was the two spotted spider mite in 2009. In those plots to where ground litter had previously been carried, specimens of *A. andersoni* could be recorded in each sampling date. The species proliferated on trees to the trunk of which ground litter had been attached, and the number of specimens on the foliage was significantly higher than in the case of control trees as early as May. Moreover, not only the treated trees but also trees in other plots were colonised from July. I got similar results in 2009.

In spite of the release, the abundance of *T. pyri* never differed significantly comparing the treated and control plots. Even if a double dosage of this predatory mite was applied, significant difference in abundance could be established in only one sampling date. The species was unable to proliferate within the plantation; the number of specimens remained around the detection threshold during the whole vegetation period. In both years, the abundance of *A. andersoni* was significantly higher than that of *T. pyri* even if both species had been released in the orchard.

b) The phytoseiid composition of the ground litter in winter following release

After the release of *A. andersoni*, the composition of phytoseiid mites in ground litter was determined the next winter. There was a significant difference in the number of *A. andersoni* between the plots; the ratio of species was 17.16% in the release plots, whereas in the control plots it was only 0.56%.

In the experimental orchard at Soroksár, I also had the opportunity to study the competition between *A. andersoni* and *E. finlandicus*. It seems that *A. andersoni* does not let the populations of its competitor increase if specimens of both species are present on the foliage. In my surveys, significantly more specimens of *A. andersoni* were recorded.

c) Phytoseiid eggs on leaves

In the case of trees colonised by *A. andersoni*, the number of eggs laid directly on leaves and that of laid onto trichomes did not show a significant difference. However, significantly more eggs were recorded from trichomes in the case of trees colonised by *E. finlandicus*.

IV. NEW SCIENTIFIC RESULTS

1. The dominance of *Typhlodromus pyri* in vineyards of the hilly wine regions of Hungary has been established.

2. 11 species (*Blattisocius tarsalis*, *Amblyseius agrestis*, *Amblyseius cucumeris*, *Amblyseius reductus*, *Amblyseius lutezhicus*, *Paraseiulus triporus*, *Anthoseius hungaricus*, *Anthoseius involutus*, *Leioseius bicolor*, *Arctoseius cetratus*, *Ameroseius pavidus*) have been collected from grapevine in Hungary for the first time. Data on the phytoseiid fauna of the wine regions of Villány, Somló, Ászár-Neszmély and Tokaj-hegyalja have firstly been presented.

3. The species *Amblyseius neobernhardi*, *Anthoseius rivulus*, *Anthoseius richteri*, *Anthoseius occiduus*, *Anthoseius pirianykae*, *Typhlodromus bichaetae*, *Blattisocius keegani* (Phytoseiidae), *Ameroseius pavidus* (Ameroseiidae), *Lasioseius fimetorum*, *Garmaniella bombophila* (Podocinidae), *Veigaia planicola* (Veigaiaidae) and *Eutogenes frater* (Cheyletidae) are new records from Hungary.

4. The new mechanical plant protection technology of tree trunk treatment with non-drying adhesive has been proved to be suitable for decreasing *Tetranychus urticae* populations on the foliage in orchards.

5. A novel method of introducing *Amblyseius andersoni* to apple orchards by using ground litter has been developed. This may be regarded as a new release technology of phytoseiid mites in plantations.

V. DISCUSSION

It is not surprising that the dominant predatory mite species is *T. pyri* in Hungarian wine regions, too, as similar data are known from several other wine growing regions of Europe. As specimens have also been collected from the ground litter during the dormancy period, I suppose that the species overwinters not only on the woody parts of the plant but also among the fallen leaves and in the upper soil level. Probably, the species had already been collected in Hungary during previous studies; however, it was identified as *T. perbibus* in several occasions. An interrelationship between predatory mite populations living in vineyards and the surrounding vegetation might be supposed based on the fact that 11 species out of the 18 phytoseiids found on different plant species were collected in the neighbouring plantations as well. In my opinion, the application of environmentally friendly plant protection technologies, which save the beneficial arthropods, can give the opportunity for phytoseiid species to colonise within plantations. In this way, the biological control of pest mites might also be solved effectively.

I prevented the crawling of *T. urticae* from the soil surface to the foliage by mechanical methods, namely making the tree trunk – the bridge between the habitats – impassable, which led to the number of specimens remaining under the damage threshold level (5 specimens/leaf) throughout the vegetation period, despite its ability to distribute with aerial dispersal. Applying non-drying adhesive on the tree trunks resulted in significant difference between the numbers of specimens in phytoseiid species, too, however, it lasted shorter than in *T. urticae*. This method might also affect other pests (woolly apple aphid and aphids), therefore I regard it as a feasible pest control method.

The novel method of introducing predatory mites to orchards by using ground litter gave convincing results in both years. So I managed to prove that (1) searching for prey, *A. andersoni* migrates from the ground litter to the canopy in spring, and (2) the ground litter is a suitable material for introducing the species to orchards. I think that a major role of applying ground litter is that it can help the proliferation of the dominant predatory mite species in newly established plantations. In my opinion, this method of releasing predatory mites with ground litter can be successful in other horticultural plants (orchards, rose, strawberry etc.), and can also serve as the means of release of other phytoseiid species which are present on the foliage in the vegetation period and overwinter in the ground litter.

I have evaluated the release of *Typhlodromus pyri* 'Mikulov' strain – common in apple orchards and vineyards – with textile strips in apple orchards. Although the release of the species was successful, the non-native strain could not proliferate in the test orchards. The reasons for this might be as follows: (1) advantage of OP-resistance of the strain could not be taken as pesticides of this group were not applied in the studied orchard; (2) specimens of the strain had been exposed to other climatic conditions than originally; (3) intraguild predation might have a negative effect on *T. pyri* populations because the species can hardly colonise apple trees in the presence of *Z. mali*, whereas this does not mean a problem in the case of *A. andersoni*; and (4) the reproductive capacity of *T. pyri* and *A. andersoni* is also different. In spite of these problems, I believe that the role of *T. pyri* in controlling phytophagous mites in apple orchards is significant. However, I would like to emphasise the difficulties of the release of the non-native species, and in relation with this, the importance of natural colonisation and of protecting the populations already present, such as in vineyards.

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