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Article

Species Composition and Seasonal Abundance of Predatory Mites (Acari: Phytoseiidae) Inhabiting *Aesculus hippocastanum*

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Abstract: Phytoseiidae inhabit a wide range of herbs, shrubs and trees. *Aesculus hippocastanum* is an important ornamental tree in Europe and is likely reservoir of these mites. We therefore assessed the species composition and the spatial and seasonal variability in the abundance of Phytoseiidae in city parks in South Bohemia, Czech Republic. Leaf samples were randomly collected from horse chestnut tree branches at eight sites, five times during the vegetation season in 2013. The mites were collected by washing technique and mounted on slides for identification. In total, 13,903 specimens of phytoseiid mites were found, and eight species were identified: *Amblyseius andersoni*, *Euseius finlandicus*, *Kampimodromus aberrans*, *Neoseiulella tiliarum*, *Phytoseilus macropilis*, *Paraseiulus talbii*, *Paraseiulus triporus* and *Typhlodromus (Typhlodromus) pyri*. *Paraseiulus talbii* and *P. macropilis* were recorded on the leaves of horse chestnut trees for the first time in the Czech Republic in this study. The predominant species was *E. finlandicus* (96.25%). The number of mites per compound leaf was, on average, 2.53, 10.40, 23.54, 11.59 and 9.27 on the sampling dates in each month between May and September, respectively. The mite density was significantly affected by the sampling site and date.

Keywords: horse chestnut tree; diversity; population dynamics; mite density; city parks

1. Introduction

The horse chestnut, *Aesculus hippocastanum* L. (Sapindales: Sapindaceae), a tree species which originated in the Epirus region and the foothills of the Pindus Mountains in northwestern Greece, has been planted in Europe since the seventeenth century primarily for ornamental purposes [1]. Many *A. hippocastanum* trees are currently grown in city parks and urban forests for their beauty, providing shade and reducing the urban heat island effect. Moreover, extracts from the various parts of this tree have been widely used in herbal medicine [2].

In addition to its importance for pollinators [3], *A. hippocastanum* is also considered an important reservoir of phytoseiid mites. A survey of Phytoseiidae on deciduous trees and bushes conducted in Finland in 1989–1991 revealed the highest density of mites to be on *A. hippocastanum*, with 1063 mites per 100 leaves on average and a maximum of 14.4 mites per leaf in a single sample [4,5]. Similar results were reported for South Bohemia, Czech Republic, where the population density of phytoseiids ranged between 1 and 28, with a mean number of mites per compound leaf of 10.5 [6]. The predominant species in both Finland and in the Czech Republic was *Euseius finlandicus* (Oudemans), which represented more than 90% of all phytoseiids found [4,6,7]. In Greece, the number of phytoseiids was found to be lower, ranging between 0 and 16, with a mean density of 4.2 mites per compound leaf; another species, *Kampimodromus aberrans* (Oudemans), was nearly as abundant as *E. finlandicus* [6].

Although the above data provide basic information on mite abundance, the spatiotemporal variability in the density of phytoseiids on *A. hippocastanum* in urban environments has not yet been studied. The objectives of the present study were therefore to investigate the species composition of

phytoseiid mites inhabiting horse chestnut trees and their abundance and its seasonal changes within a medium-size city.

2. Materials and Methods

2.1. Sampling sites

The research was carried out in the town of České Budějovice, Czech Republic (48° 59' N, 14° 29' E; 386 m above sea level). The town, which is predominantly surrounded by forests and agricultural fields, has about 260 hectares of open public green space inhabiting 534 horse chestnut trees [8]. Eight sampling sites were defined within the cadastral area of the town (Table 1).

Table 1. Characteristics of *Aesculus hippocastanum* sampling sites within České Budějovice.

Local name	Geographical coordinates of geometric centre	<i>A. hippocastanum</i> age in years (mean ± SE) ¹
City centre	48.9744136N, 14.4770594E	74.99 ± 1.86
Šumava and Máj estate	48.9841631N, 14.4407714E	39.02 ± 3.19
Vltava estate	48.9962106N, 14.4519647E	41.79 ± 3.49
Třebotovice and Kaliště village	48.9612606N, 14.5651828E	17.82 ± 6.86
Rožnov estate	48.9601436N, 14.4763944E	70.49 ± 4.43
Pražské předměstí estate	48.9860569N, 14.4674681E	49.74 ± 2.75
Stromovka Park	48.9670208N, 14.4551158E	43.53 ± 4.13
Nádražní Street	48.9779942N, 14.4866511E	77.74 ± 3.02

¹ Tree age was estimated by method proposed by Jura [9].

2.2. Sampling of horse chestnut leaves

The population of Phytoseiidae was assessed on horse chestnut leaves that were collected randomly five times during the vegetation season in 2013. The sampling dates were from 16th to 24th May, from 13th to 21st June, from 11th to 19th July, from 11th to 22nd August and from 9th to 19th September. The sampling took place only when there had been no rain for at least 48 hours prior to sampling. A randomly selected compound leaf collected from *A. hippocastanum* tree up to 2.5 m above ground represented the sample unit. In total, 30 leaves were collected per sampling site. The sampled trees were selected evenly across the whole site. Only one leaf per tree was collected except at sites with less than 30 trees. The leaves at each individual site were all sampled on the same day, placed individually into polyethylene bags and transported in cool box to the laboratory where the leaves were stored at temperature 4°C for less than 24 hours before they were processed.

2.3. Collection and identification of phytoseiid mites

The mites were collected from individual leaves using the washing technique [10]. After taking the photographs, the horse chestnut leaf was held by the petiole, and individual leaflets were cut off by pruning shears into a glass jar (volume 700 ml) containing 350 ml of 85% ethanol. The jar was closed carefully and shaken vigorously for two minutes. Afterwards, each leaflet was removed by tweezer and washed with ethanol using a plastic wash bottle. Particular attention was paid to trichomes and domatia during washing.

The contents of the glass jar were poured through a plastic funnel into a glass dividing funnel with a Teflon® stopcock. The empty glass jar and the plastic funnel were washed with 85% ethanol, which was poured into the dividing funnel immediately. All invertebrates settled at the bottom of the dividing funnel within 5 minutes. Then, the Teflon® stopcock was opened, and approximately 50 ml of ethanol sample containing all invertebrates was poured off into a small glass vial with a plastic plug. The material was stored in this vial until microscope slide preparation and identification.

Each vial with preserved mites was placed in a paper holder to prevent spillage, and the ethanol in the vials was gradually transferred onto a watch glass by using a plastic Pasteur pipette. The

sample on the watch glass was inspected using a dissection microscope. All mites were removed with a wire loop and mounted on temporal microscope slides in lactic acid. The mites were identified using the keys of [11–15].

2.4. Data presentation and statistical analysis

The abundance of phytoseiid mites was expressed as the number of mites per compound *A. hippocastanum* leaf and analyzed by GLMM using a Poisson distribution, and log-link function. The analysis was performed in SAS® Studio for Linux using the GLIMMIX procedure of SAS/STAT® module [16].

The species diversity was quantified using Simpson's diversity index [17]: $D=1-((\sum_i n_i(n_i-1))/(N(N-1)))$, where n is the total number of organisms of a particular species, and N is the total number of organisms of all species. The value of the index ranges between 0 and 1, and the greater the value is, the greater the species diversity. The coefficient of constancy (C) [18] was used to indicate the frequency of different species in the studied localities: $C(\%)=N_a/N \times 100$, where N_a is the number of samples with species a , and N is the total number of samples. The species were classified as accidental ($C<25\%$), accessory ($C=25-50\%$), constant ($C=50-75\%$) or euconstant ($C>75\%$) [18].

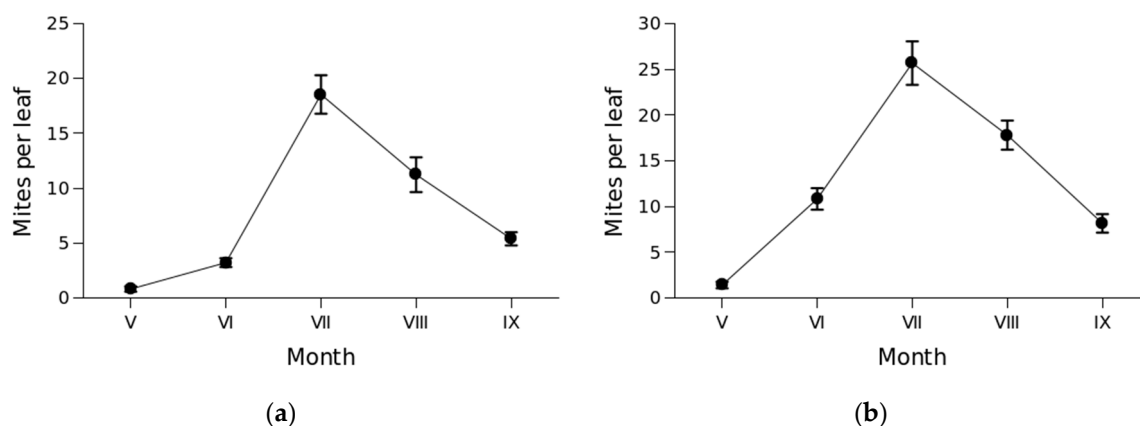
3. Results

3.1. Species composition

A total of 13,903 phytoseiid mites belonging to eight species were identified. The species composition was as follows: *E. finlandicus* 96.25% ($C=82.33\%$), *Typhlodromus* (*Typhlodromus*) *pyri* Scheuten 1.35% ($C=10.00\%$), *Amblyseius andersoni* (Chant) 0.80% ($C=4.50\%$), *Neoseiulella tiliarum* (Oudemans) 0.73% ($C=5.00\%$), *K. aberrans* 0.58% ($C=5.83\%$), *Phytoseiulus macropilis* (Banks) 0.22% ($C=1.00\%$), *Paraseiulus triporus* (Chant & Yoshida-Shaul) 0.06% ($C=0.33\%$) and *Paraseiulus talbii* (Athias-Henriot) 0.01% ($C=0.08\%$). According to the coefficient of constancy, all species can be considered accidental except *E. finlandicus*, which is a euconstant species. Simpson's diversity index was only 0.0733.

3.2. Mite abundance and seasonal dynamics

The mean abundance of phytoseiid mites per compound horse chestnut leaf across all sites was 2.53 (SE=0.292; $n=240$), 10.40 (SE=0.512; $n=240$), 23.54 (SE=0.909; $n=240$), 11.59 (SE=0.492; $n=240$) and 9.27 (SE=0.491; $n=240$) in May, June, July, August and September, respectively. The overall annual average abundance was 11.47 mites per leaf (SE=0.324, $n=1200$). Population density of Phytoseiidae and its seasonal changes differed among sampling sites (Figure 1). The statistical analysis confirmed significant effect of site ($F_{7,1160}=99.76$; $P<0.0001$). The abundance of mites fluctuated over the season ($F_{4,1160}=869.16$; $P<0.001$) and the interaction between site and time was also significant ($F_{28,1160}=38.18$; $P<0.001$).



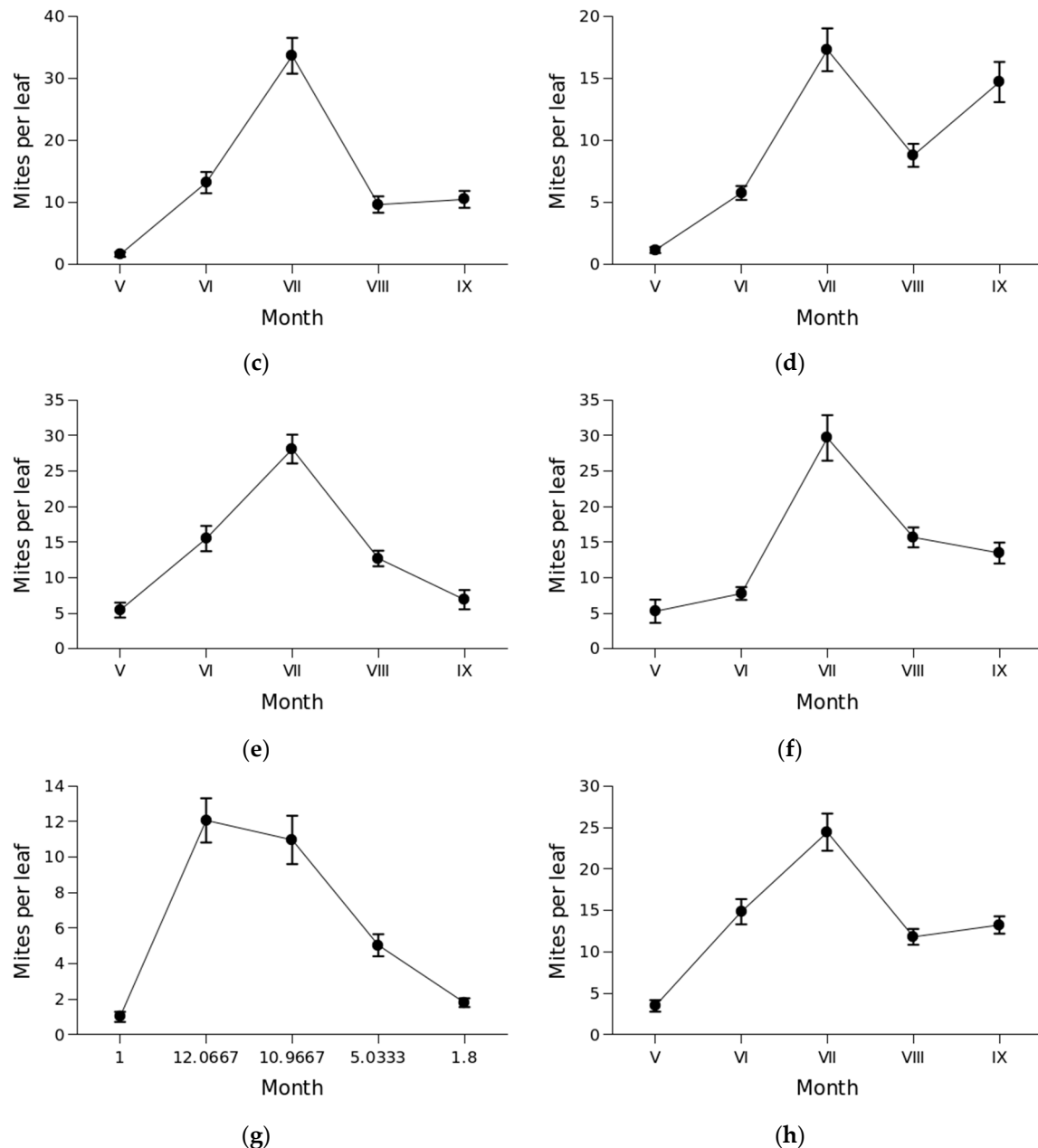


Figure 1. The mean abundance of phytoseiid mites per *Aesculus hippocastanum* leaf during vegetation period at eight sampling sites in České Budějovice. (a) City centre; (b) Šumava and Máj estate; (c) Vltava estate; (d) Třebotovice and Kaliště village; (e) Rožnov estate; (f) Pražské předměstí estate; (g) Stromovka park; (h) Nádražní street. Vertical bars indicate standard error of the mean (n=30).

4. Discussion

The Phytoseiidae family represents a very important group of predatory mites that inhabit, in addition to herbaceous plants, many species of deciduous trees and shrubs [4,5,19–31]. Six species of phytoseiids were identified on horse chestnut in city parks in Prague, Czech Republic, by Kabíček and Řeháková [7]: *E. finlandicus*, *Galendromus longipilus* (Nesbitt), *K. aberrans*, *Neoseiulella aceri* (Collyer), *N. tiliarum* and *T. (T.) pyri*. The species richness, however, varied among the investigated sites, from one to four species.

The species composition of the present study confirmed the presence of these species, except *G. longipilus* and *N. aceri*, which were not found in any sample in České Budějovice. However, we found these other species: *A. andersoni*, *Ph. macropilis*, *Pa. triporus* and *Pa. talbii*. Thus, eight species were found on *A. hippocastanum* in total. Six of them had also been found on horse chestnut in Hungary

[32,33], while *Pa. talbii* and *Ph. Macropilis*, which were identified in the present study, have not yet been reported on horse chestnut leaves. In Greece, where horse chestnut is an autochthonous tree, only four phytoseiid species were found: *E. finlandicus*, *K. aberrans*, *Pa. talbii* and *T. (T.) pyri* [6]. The reason for the lower number of species might be the much less intensive sampling compared to that used in the present study.

The most abundant species found in the present study was *E. finlandicus*, representing approximately 96% of all phytoseiid mites. This confirms that *E. finlandicus* is predominant in species complexes of phytoseiid mites in Europe on horse chestnut [4,7,27]. In Greece, however, the percentage of *E. finlandicus* on *A. hippocastanum* was found to be only 48%, and the second most dominant species, representing approximately 43% of all determined species of Phytoseiidae, was *K. aberrans* [6]. *Euseius finlandicus* is also the predominant species on other deciduous trees [4,5,21,24,25,27,34]. For example, in a survey by Kabíček and Povondrová [24], *E. finlandicus* represented more than 95% of all phytoseiid mites identified in leaf samples collected from various deciduous trees in a park in Prague.

The results of the present study revealed that one *A. hippocastanum* leaf can host 2.5 - 23.5 phytoseiid mites on average depending on the month. These abundance values agree with those of previous studies conducted in Finland [4,5] and the Czech Republic [7]. The latter authors reported the highest population density of phytoseiids on horse chestnut trees in Prague to be 3.3 mites per leaflet on average [7]. Because *A. hippocastanum* compound leaves usually have five to seven leaflets [1], the above value could correspond to 19.8 mites per average compound leaf. This result matches that of our third sampling in July. In contrast, the density of phytoseiid mites in Greece was lower, with 4.2 mites per compound leaf on average [6].

Although phytophagous mites are a common food source for most Phytoseiidae [20], we found only a few phytophagous mites in our samples. Among the mites infesting the horse chestnut tree are *Eotetranychus pruni* (Oudemans) (Acari: Tetranychidae) [35], *Aculus hippocastani* (Fockeu) and *Shevtchenkella carinatus* (Nalepa) (Acari: Eriophyidae) [36]. However, according to Tuovinen and Rokx [4], prey density does not seem to have any significant effect on the presence and density of *E. finlandicus* or *P. macropilis*. The number of phytoseiids on leaves is influenced by leaf surface characteristics rather than by food availability [37]. Horse chestnut trees have few glandular trichomes with a mean height of 84 µm located only on the midrib surface on the adaxial epidermis; nonglandular trichomes with lengths ranging from 116-436 µm were observed on the lower leaf surface, where they were located on the midrib and lateral veins as well as in the vein axils [38]. The density of leaf trichomes was reported to be 9.96/mm², which is relatively high compared to that in other tree species [39]. The relatively high phytoseiid density on *A. hippocastanum* can thus be explained by the favorable micromorphology of its leaves. A positive effect of leaf trichomes and domatia occurrence on the abundance of predatory mites has been well documented in many studies [21,22,37,40–50]. While domatia mainly provide phytoseiid mites with shelter and act as protection from either natural enemies or abiotic stress [42], leaf pubescence also increases the capture and retention of pollen and fungal spores that serve as alternative foods [51]. We often observed many pollen grains on *A. hippocastanum* leaves. Kugler [52] estimated that this tree species itself can produce 42 million pollen grains from a single inflorescence. The *A. hippocastanum* pollen was also found to have a very high nutritional quality for phytoseiid mites [53,54]. Because the highest pollen concentration occurs during May [55], its availability probably facilitated the significant increase in phytoseiid mite density in June and July observed in our study. The other food resources like extrafloral nectar or fungi are also considered to be important for generalist phytoseiid mites [56,57] and their role in nutrition of mites inhabiting horse chestnut needs to be investigated, too.

5. Conclusions

The present study confirmed that (1) the horse chestnut tree is a favorable host tree for phytoseiid mites, (2) the density of mites varied significantly between sites and months, with its peak density in July, and (3) *E. finlandicus* was the predominant species. Two species, *P. talbii* and *P. macropilis* were recorded on horse chestnut for the first time.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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