

Diversity and abundance of Phytoseiidae (Acari: Mesostigmata) on horse chestnut (*Aesculus hippocastanum* L.) in an urban environment: a comparison between Greece and the Czech Republic

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ABSTRACT

Horse chestnut, *Aesculus hippocastanum* L. (Sapindaceae), is a large deciduous tree native to a small area in the Pindus Mountain mixed forests and the Balkan mixed forests of Southeast Europe. It was introduced to most countries in Europe mainly for ornamental purposes. The aim of the present study was to assess the occurrence, species composition and population densities of phytoseiid mites on *A. hippocastanum* in countries where this tree species is autochthonous and to compare them with Central Europe. In addition, we tested whether the horse chestnut leaf miner, *Cameraria ohridella* or the horse chestnut leaf blotch, *Guignardia aesculi* has any effect on Phytoseiidae population density. Leaf samples were collected in the cities of České Budějovice, Czech Republic and Orestiada, Greece in September 2013 and 2015, respectively. Thirty compound leaves were randomly taken from tree branches up to 2.5 m above ground in both localities. Mites were collected by washing the leaves in ethanol and were then mounted in lactic acid and identified. A total of 441 specimens of phytoseiid mites belonging to six species (*Euseius finlandicus*, *Neoseiulella tiliarum*, *Kampimodromus aberrans*, *Paraseiulus talbii*, *Phytoseius macropilis* and *Typhlodromus (Typhlodromus) pyri*) were collected. A significantly higher population density of Phytoseiidae was found in České Budějovice. The density was not affected by the horse chestnut leaf miner, *Cameraria ohridella* or the horse chestnut leaf blotch, *Guignardia aesculi*. *Euseius finlandicus* was the predominant species in both České Budějovice (96.8%) and Orestiada (48.4%) where, however, it competed with *K. aberrans* (42.9%).

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Introduction

Horse chestnut, *Aesculus hippocastanum* L. (Sapindaceae), is a large deciduous tree native to a small area in the Pindus Mountain mixed forests and the Balkan mixed forests of Southeast Europe. It was introduced to most countries of Europe for mainly ornamental purposes. Its ornamental function has been negatively affected by the horse chestnut leaf miner, *Cameraria ohridella* Deschka & Dimic (Lepidoptera: Gracillariidae), in the last two decades. This species was recorded at the Ohrid Lake in F.Y.R.O.M. in 1985 for the first time and became an invasive pest when it spread through Austria to the entire territory of Europe and Asia Minor. In the Czech Republic, this species appeared in 1993 (Liška, 1997). This species may damage up to 93% of the leaf area in an urban environment. Another cause of mostly aesthetic damage is the horse chestnut leaf blotch, *Guignardia aesculi* (Peck) V.B. Stewart (Botryosphaeriales: Botryosphaeriaceae). This fungal disease was uncovered in the Czech Republic in the 1950s (Scaramuzzi 1954) and has been reported in other European countries, the USA and Asia (Pastirčáková *et al.*, 2009).

In addition to the ornamental function of *A. hippocastanum*, this tree might also serve as an important reservoir of predatory mites of the family Phytoseiidae (Acari: Mesostigmata) which spread to apple orchards or vineyards (Tuovinen and Rokx, 1991; Tuovinen, 1994). While a survey of phytoseiid mites on this tree species has already been conducted in the Czech Republic (Kabíček and Řeháková, 2004), and some data have also been reported for Finland (Tuovinen and Rokx, 1991), no such study has been conducted in a country where horse chestnut is an autochthonous tree. Therefore, the aim of the present study was to compare the occurrence, species composition and population densities of Phytoseiidae on introduced *A. hippocastanum* in Central Europe with that on the tree species in Greece. Since some phytoseiid species feed on fungi (Zemek and Prenerová, 1997; Duso *et al.*, 2003; Zemek, 2005; Pozzebon *et al.*, 2009), we hypothesize that the population density of phytoseiids might be positively correlated with the occurrence of the fungal pathogen *G. aesculi*. A higher infestation by *C. ohridella* might also support a phytoseiid population by providing more shelters similar to abandoned mines of the citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) (Villanueva and Childers, 2011). Therefore, we also tested whether *G. aesculi* and *C. ohridella* had any effect on the population density of phytoseiid mites.

Materials and methods

Study sites

The survey was conducted in the cities of České Budějovice, South Bohemia, Czech Republic (48° 59' N, 14° 29' E) and Orestiada, northeastern Greece (41° 30' N, 26° 32' E). České Budějovice has a population of approximately 100,000 inhabitants and an urban area of 55 km² with the total number of *A. hippocastanum* trees in the town estimated to be 534. The trees are located mostly in city parks, green belts along rivers and streams and open space (Kopačka and Zemek, 2017). Orestiada is a smaller city with a population of approximately 20,000 inhabitants and an urban area of 39 km² with approximately 83 *A. hippocastanum* trees, mostly found in alleys. Both towns are surrounded by agricultural fields, grasslands and woods.

In České Budějovice, thirty horse chestnut trees were randomly selected at the Vltava estate site (area approx. 92 ha, geometric center at 48.9962106N, 14.4519647E) for the purpose of this study. The mean age of horse chestnut trees at this site, estimated from the trunk perimeter using the method described by Jura (2001), was 41.8 years (Kopačka and Zemek, 2017). In Orestiada, *A. hippocastanum* trees were randomly selected in an area of approx. 130 ha with a geometric center at 41.5071667N, 26.5295267E. The estimated mean age of the trees was 28.2 years.

Sampling

The population of Phytoseiidae was assessed on thirty randomly selected compound leaves collected from tree branches up to 2.5 m above ground. Only one compound leaf from a single tree was collected. The sampling was conducted on 13th September 2013 and 30th September 2015 in České Budějovice and Orestiada, respectively.

First, the percentage of leaf area damaged by *C. ohridella*, *G. aesculi* or another cause, e.g., bird predation on *C. ohridella* larvae and pupae, or mechanical damage from weather-related events was estimated by visual method (Gilbert and Grégoire, 2003). Then, all mites were collected from individual leaves using a washing technique (Zacharda et al., 1988). This was done by splitting each compound leaf into individual leaflets, which were in turn placed in a sealable glass jar with 85% ethanol in which mites were washed off. The collected mites were stored in small glass vials (one vial per leaf sample) with 85% ethanol and then mounted on temporal microscope slides in lactic acid. The mites were identified using the keys of Beglyarov (1981a,b) and Miedema (1987).

Data analysis

All data were stored in a Microsoft Access 2010 database. The abundance of phytoseiid mites was expressed as the mean number of mites per composed *A. hippocastanum* leaf. The species diversity was quantified using Simpson's Diversity Index (Adams, 2009):

$$D = 1 - ((\sum_i n_i(n_i - 1)) / N(N - 1)),$$

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; the greater the value, the greater the species diversity. The Sørensen's index (QS) or similarity coefficient (Southwood, 1968) was used to evaluate β -diversity: $QS = 2j/(a+b)$, where a is the number of species in locality A, b the number of species in the locality B and j is the number of common species in both localities; the value of the index ranges between 0 and 1. The coefficient of constancy (C) (Dajoz, 1977) 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 the 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 ($C > 75\%$) (Dajoz, 1977). The obtained leaf damage data were statistically analyzed using the Mann-Whitney U test (Siegel and Castellan, 1988). The significance was set at $\alpha = 0.05$ with the Holm-Bonferroni correction applied for multiple comparisons (Holm, 1979). The abundance of phytoseiid mites was analyzed by ANCOVA with location as a main factor and the leaf damage inflicted by *C. ohridella* and *G. aesculi* and mechanical damage as continuous predictors. Two-tailed probabilities were used. All statistical analyses were performed by STATISTICA v. 12 software (StatSoft Inc., 2013).

Table 1 Leaf damage inflicted to *Aesculus hippocastanum* leaves in České Budějovice and Orestiada.

Cause of damage	České Budějovice	Orestiada	Mann-Whitney U test	
	(mean±SE)	(mean±SE)	Raw P	Significance ^a
<i>Cameraria ohridella</i>	3.53±0.43	4.20±1.80	0.048	NS
<i>Guignardia aesculi</i>	6.73±0.84	4.23±0.85	0.005	*
Other cause	2.00±0.32	1.40±0.50	0.03	NS

^a Significant differences at $\alpha = 0.05$ adjusted according to the Holm-Bonferroni method for multiple comparisons (Holm, 1979).

Results

The estimated percentage of leaf area damaged by *C. ohridella*, *G. aesculi* or other causes in České Budějovice ranged from 0% to 10%, 2% to 25%, and 0% to 5%, respectively. The highest mean leaf damage (6.73%) was caused by the fungal pathogen (Table 1). The leaf damage in Orestiada ranged from 0% to 50%, 0% to 25% and 0% to 10% for *C. ohridella*, *G. aesculi* and other causes, respectively. On average, the damaged leaf area was 4.20%, 4.23% and 1.40%, respectively. The Mann-Whitney U test revealed statistically significant differences between localities in damage inflicted by *G. aesculi* (Table 1).

A total of 441 specimens of phytoseiid mites were collected from both countries. Phytoseiidae were found in all leaf samples in České Budějovice. The population density of mites ranged between 1 and 28, and the mean number of mites per leaf was 10.5. In contrast, in Orestiada, 10% of the samples were devoid of phytoseiid mites; the maximum number of mites in a sample was 16 mites, and on average, there were 4.2 phytoseiid mites per leaf. The ANCOVA revealed a highly significant effect of locality on phytoseiid mite abundance ($F_{1,55}=15.973$, $P=0.0002$). The effects of covariates were, however, not statistically significant ($F_{1,55}=0.029$, $P=0.865$; $F_{1,55}=0.853$, $P=0.360$ and $F_{1,55}=0.367$, $P=0.547$) for the leaf damage caused by *C. ohridella*, *G. aesculi* or other causes, respectively.

The following five species of phytoseiid mites were found in České Budějovice: *Euseius finlandicus* (Oudemans), *Kampimodromus aberrans* (Oudemans), *Neoseiulella tiliarum* (Oudemans), *Phytoseius macropilis* (Banks) and *Typhlodromus* (*Typhlodromus*) *pyri* Scheuten (Table 2). While *E. finlandicus* was present in all samples ($C=100\%$), *K. aberrans* and *N. tiliarum* were found in only one sample ($C=3.33\%$). In Orestiada, samples were devoid of *N. tiliarum* and *P. macropilis*, while another species, *Paraseiulus talbii* (Athias-Henriot), was found in two samples ($C=6.67\%$). This species composition resulted in rather high similarity ($QS=0.667$) between studied sites. The predominant species in České Budějovice was *E. finlandicus*, representing 96.8% of all specimens (Figure 1). In Orestiada, the following two species were predominant: *E. finlandicus* (48.4%) and *K. aberrans* (42.9%). The Simpson's Diversity Index was therefore higher for Greece (0.583) than for the Czech Republic (0.062).

Discussion

Our study revealed a high population density of Phytoseiidae on *A. hippocastanum*, particularly in the Czech Republic where there were more than ten mites per composed leaf; i.e., approximately two mites per single leaflet were found. Kabíček and Řeháková (2004) reported the

Table 2 Abundance (mean number $\pm$ SE of mites per composed leaf) and the coefficient of constancy (%) of Phytoseiidae on *Aesculus hippocastanum* in České Budějovice and Orestiada.

Species	České Budějovice		Orestiada	
	Mites/leaf	C^a	Mites/leaf	C^a
<i>Euseius finlandicus</i>	10.17 $\pm$ 1.36	100	2.03 $\pm$ 0.66	43.33
<i>Phytoseius macropilis</i>	0.17 $\pm$ 0.08	13.33	-	-
<i>Typhlodromus pyri</i>	0.10 $\pm$ 0.06	10	0.23 $\pm$ 0.09	20
<i>Kampimodromus aberrans</i>	0.03 $\pm$ 0.03	3.33	1.80 $\pm$ 0.36	66.67
<i>Neoseiulella tiliarum</i>	0.03 $\pm$ 0.03	3.33	-	-
<i>Paraseiulus talbii</i>	-	-	0.13 $\pm$ 0.09	6.67
Total	10.50 $\pm$ 1.37		4.20 $\pm$ 0.68	

^a Coefficient of constancy; see Materials and Methods for its calculation.

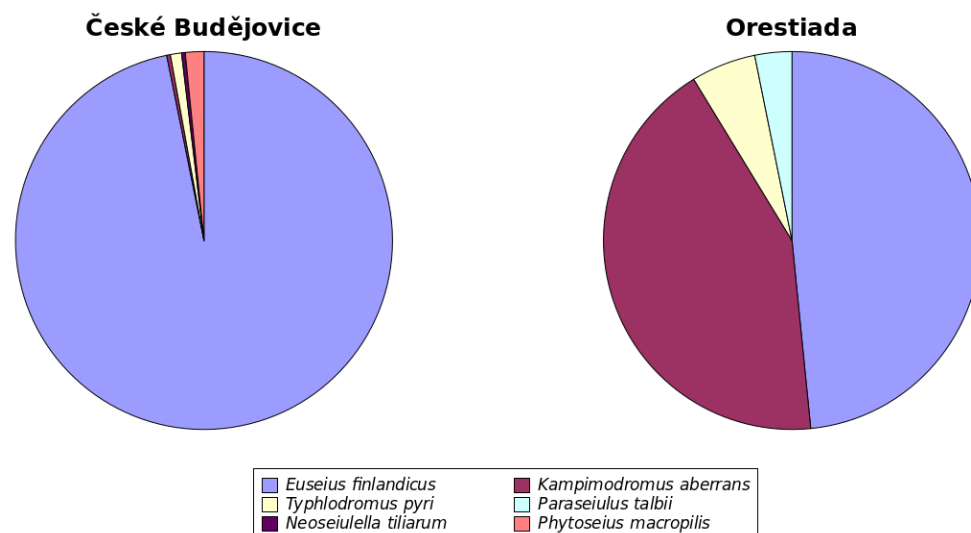


Figure 1 Species composition of Phytoseiidae on *Aesculus hippocastanum* in České Budějovice (left) and Orestiađa (right).

highest population density of phytoseiids on horse chestnut trees to be 3.3 mites per leaflet on average at the Prokopské údolí site, and the lowest to be 2.5 mites per leaflet at the Letenské sady site in Prague. The abundance of phytoseiid mites on *A. hippocastanum* in Orestiađa was significantly lower, which might be attributed to prey availability, different abiotic conditions or less greenery in the city making the trees more isolated. In regard to prey associated with *A. hippocastanum*, Ripka and de Lillo (1997) reported *Aculus hippocastani* (Fockeu) and *Shevtchenkella carinatus* (Nalepa) (Acari: Eriophyidae), and Gyenis et al. (2005) reported *Eotetranychus pruni* (Oudemans) (Acari: Tetranychidae), infesting horse chestnut. Only a few phytophagous mites were collected in the samples from České Budějovice. Sampling efforts in the present study, however, did not focus on the eriophyid fauna. Availability of alternative food, e.g., pollen or fungi on leaves, might be another reason for the above differences. Aspects of the host plant are also considered to influence the behavior and distribution of phytoseiids independently of prey availability (Beard and Walter, 2001). The presence of leaf structures such as vein trichomes and leaf domatia is known to have a positive effect on the number of microarthropods, including phytoseiid mites (O'Dowd and Willson, 1989; Walter and O'Dowd, 1992; Karban et al., 1995; Kreiter et al., 2002; Schmidt, 2014), but no apparent differences in these leaf structures between the studied localities were seen by the naked eye.

We were also interested in whether there was any effect of *C. ohridella* or *G. aesculi* infestation on the abundance of Phytoseiidae. There could be either a negative effect due to leaf damage, thus reducing leaf area for herbivorous mites, or a positive effect when, for example, *C. ohridella* eggs could serve as an alternative food source. Grabenweger et al. (2005) studied whether *E. finlandicus* is able to utilize these eggs as a food source but observed no feeding. Mine-damaged *A. hippocastanum* leaves can also provide refugia to phytoseiid mites with more favorable microenvironment conditions than leaves without *C. ohridella* damage. In fact, they may function in a similar way to domatia. For example, in apples, Villanueva and Harmsen (1996) observed the phytoseiids *Typhlodromus caudiglans* Schuster and *Neoseiulus fallacis* (Garman) in old mines produced by the spotted tentiform leaf miner, *Phyllonorycter blancardella* Fabricius (Lepidoptera: Gracillariidae). A positive effect of the infestation by a leaf miner on the population of phytoseiid mites was reported by Villanueva and Childers (2011) who found that Phytoseiidae were significantly more abundant on mature grapefruit leaves mined by *P. citrella* than on mature leaves without mines. Our results, however, did not confirm

such an effect. The leaf area damaged by the leaf blotch was significantly higher in České Budějovice than that in Orestiada, which is likely due to favorable abiotic conditions, mainly humidity. Since fungivory in some phytoseiid species was well-documented, for example *T. pyri*, *E. finlandicus* and *K. aberrans* readily feed on several mildew species (Kropczyńska-Linkiewicz, 1971; Zemek and Prenerová, 1997; Duso et al., 2003; Zemek, 2005; Pozzebon et al., 2009), we expected that they could utilize *G. aesculi* as a food source as well. No correlation between mite density and the leaf blotch damage was, however, confirmed in this study.

Five species were identified among specimens in České Budějovice and four in Orestiada, while three species were common in both sites. This resulted in a relatively high Sørensen's index indicating certain similarity between sites. Kabíček and Řeháková (2004) reported six phytoseiid species in total on *A. hippocastanum* found in Prague, but the species richness varied among investigated sites from one to four species. The authors claim that this variability may be determined by the different locality conditions in the city. Contrary to the present study, *P. macropilis* was not present; however, *Galendromus longipilus* (Nesbitt) and *Neoseiulella aceri* (Collyer) were found. Six species of the Phytoseiidae were found on horse chestnut in Hungary (Ripka, 2006). They include two species which were not present in our samples: *Amblyseius andersoni* (Chant) and *Paraseiulus triporus* (Chant and Yoshida-Shaul). The most numerous species found in both České Budějovice and Orestiada was *E. finlandicus*. This species was found on 68% of surveyed trees in the park of the Czech Agricultural University, Prague (Kabíček and Koubková, 1998), while it represented approximately 96% of all specimens found on investigated deciduous trees in the Prague urban forest at Kunratice (Kabíček and Povondrová, 2004). *E. finlandicus* was also the predominant species on *A. hippocastanum* where it represented almost 98% of all phytoseiid specimens found (Kabíček and Řeháková, 2004), which is very close to our results. In Greece, however, another species, *K. aberrans*, is nearly as abundant as *E. finlandicus*; therefore, the Simpson's diversity index, which takes into account both species richness and evenness, was higher in Greece than in the Czech Republic. We hypothesize that this is mainly due to different climate conditions.

We can conclude that (1) horse chestnut trees represent favorable host plants for phytoseiid mites with *E. finlandicus* as the predominant species in both countries, (2) higher abundance of Phytoseiidae on *A. hippocastanum* but lower Simpson's diversity index was found in the Czech Republic compared to Greece, and (3) no significant effect of horse chestnut leaf miner or leaf blotch on population density of phytoseiid mites was confirmed.

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