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ORIGINAL ARTICLE

Effect of sun-exposure of the horse chestnut (*Aesculus hippocastanum* L.) on the occurrence and number of parasitoids of the horse chestnut leafminer (*Cameraria ohridella* Deschka & Dimic) in central Poland in 2004–2006

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Abstract

The research on parasitoids of the horse chestnut leafminer (*Cameraria ohridella* Deschka & Dymić, 1986) of the order Hymenoptera was conducted in the years 2004–2006 in six locations in central Poland. The complex of parasitoids was composed of 14 species. *Minotetrastrichus frontalis* (Nees) and *Pnigalio agraules* (Walker), (Hymenoptera: Eulophidae) were the dominant species in all locations. It was noted that sun-exposure of the horse chestnut (*Aesculus hippocastanum* L.) had a significant effect on abundance of parasitoids attacking the horse chestnut leafminer and their percentage share over the study period. The greatest number of parasitoids was found in the trees which were most exposed to sunray penetration, thus the warmest.

Key words

Cameraria ohridella, tree sun-exposure, parasitoids, *Minotetrastrichus frontalis*, *Pnigalio agraules*

Introduction

Over the last dozen or so years, leaves of the horse chestnut (*Aesculus hippocastanum* L., Hippocastanaceae) have been attacked in Europe by the horse chestnut leafminer *Cameraria ohridella* Lepidoptera: Gracillariidae (Deschka & Dymić, 1986). Natural enemies usually play a crucial role in leafminer population control (Askew and Shaw 1979). Intensive research on parasitoids damaging horse chestnut leafminers has shown that about 20 species of the order Hymenoptera develop on larvae or pupae of *C. ohridella* (Maier 1984; Grabenweger 1998; Grabenweger and Lethmayer 1999; Stojanović and Marković 2004). Most of them are chalcid wasps (Chalcidoidea) belonging to the family Eulophidae, less numerous are giant ichneumonids (Ichneumonidea) and braconids (Braconidae) (Grabenwerger 1998). The parasitoids damaging horse chestnut leafminers are in general polyphages adapting to the new host (Grabenweger and Lethmayer 1999).

A level of parasitism of horse chestnut leafminers varies from several to a dozen or so percent (Stolz since 1997; Grabenweger and Lethmayer 1999; Freise and Heitland 2001; Freise et al. 2002; Kehrli and Bacher 2003; Boisneau et al. 2004; Volter 2004; Stojanović and Marković 2004). This variation may be the result of ap-

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plication of different methods of assessments or difficulties in prcise estimation of phytophage or parasitoid population numbers (Grabenweger and Lethmayer 1999; Grabenweger et al. 2005; Grabenweger 2003). Assessment of the number of mines on horse chestnut leaves, without leaf detailed vivisection, is possible exclusively for horse chestnut leafminer first generation. Each next generation developing in successive weeks makes assessments more difficult. With the development of next generations, the number of mines increases, however many of them become 'empty' as moths and parasitoids leave them. Therefore, counting of mines without leaf vivisection is of low usefulness or even misleading. The variability of parasitism percentage may also depend on certain features of habitats from which come colonized leaves.

The main purpose of the study is to show variability of parasitism levels in the *C. ohridella* population depending on the degree of sun-exposure of *A. hippocastanum*.

Material and methods

Horse chestnut leaves colonized by *C. ohridella* were collected in the years 2004–2006, in autumn – after leaf fall. The material came from six different locations in central Poland (Fig. 1, Tab. 1). The habitats from which leaves were collected differed in a degree of sun-exposure (intensely insolated, moderately insolated, very much shaded) as well as the composition of tree species in close vicinity of the leaf collection area. The leaf samples were exposed to low temperatures under field conditions from December to February. Next, the leaf samples were placed in photoeclectors.

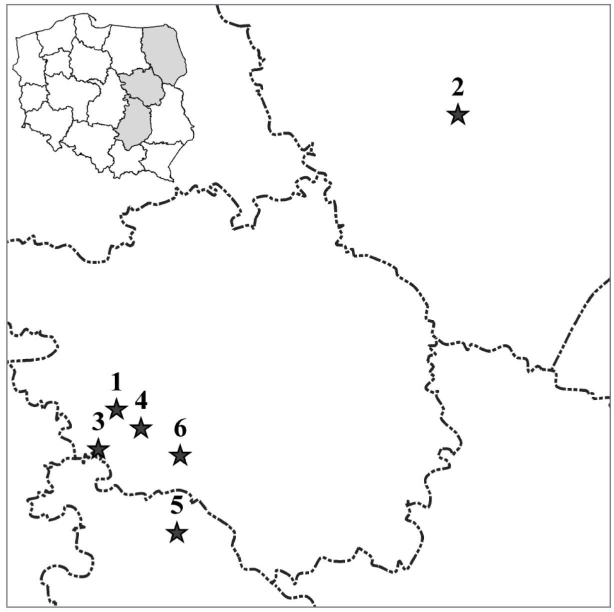

Fig. 1. Location of trees from which leaves were collected

Each photoeclector was a 56 × 42 × 35 cm cardboard box with a small hole, where plastic 150 ml container

Tab. 1. Description of the location of the experimental trees

Localisation number	Location	Coordinate	Characteristics
No. 1	Koło	52° 14' 55" N 20° 56' 39" E	A mid-forest horse chestnut avenue, moderately shaded, 1–2 m from the crowns of high trees (maples, lindens, oaks).
No. 2	Stelmachowo	53° 09' 34,5" N 22° 44' 56,9" E	A roadside horse chestnut avenue, intensely insolated, in mid-field, 1–2 m from the crowns of high trees (ashes, maples, lindens).
No. 3	Osowiec	52° 02' 06" N 20° 39' 54" E	A roadside horse chestnut avenue, intensely insolated, surrounded by buildings. 50–100 m from the crowns of high trees (maples, ashes, false acacias).
No. 4	Falenty	52° 08' 20" N 20° 55' 32" E	A roadside horse chestnut avenue, intensely insolated, surrounded by fields. 10–20 m from the crowns of high trees (ashes, maples, lindens).
No. 5	Protected stand "Modrzewina"	51° 50' 00" N 20° 47' 01" E	A mid-forest horse chestnut avenue, greatly shaded. 1–2 m from the crowns of high trees (larches, oaks, maples, pines).
No. 6	Obory	52° 05' 2,7" N 21° 08' 33" E	Single roadside horse chestnut trees, intensely insolated, in the surrounding of the park. 5–10 m from the crowns of high trees (ashes, maples, lindens).

filled with glycol was placed. The investigated material was placed in photoeclectors and reared at temperature 22 ± 1° C and humidity 60 ± 5%. Observed samples contained 100 single laminas of composite leaves of the studied horse chestnut trees. Hatching insects were collected from the plastic containers every two weeks until flying out was completed. Then horse chestnut leafminer moths were counted and their parasitoids identified and also counted (Grabenweger 1998). After captures were completed, dried leaves collected from photoeclectors were weighed and the average number of *C. ohridella* moths and parasitoids per 100 g dry mass of leaves was assessed. The level of parasitism was determined as WPS (relative parasitism percentage) according to the formula:

$$\text{WPS} = \frac{\text{Number of reared parasitoids} \times 100}{\text{Number of reared moths} + \text{Number of reared parasitoids/sample}} \ [\%]$$

The above formula is a modification of the one used by Grabenweger and Lethmayer (1999) to determine the level of parasitism which is assessed by the number of preimaginal development stages obtained with the use of the vivisection method in place of using the number of moths and parasitoids reared from 100 g of horse chestnut dry leaves.

For statistical analysis purposes, single-factor analysis of variance (ANOVA) was applied using the program Statgraphics®*Plus* for Windows 4.1 (Copyright© 1994–1999 by Statistical Graphics Crop., USA) and homogenous groups were tested with Tukey's Test.

Results

During the three-year period of insect rearing 11197 moths and 4616 parasitoids were obtained. Fig. 2 illustrates the total number of all moths and parasitoids collected in individual locations as assessed per 100 g of leaf dry mass. The largest number of parasitoids flew out from horse chestnut leaves collected in location 6 (Obory, intensely insolated), the lowest – in location 5 (Protected stand "Modrzewia", greatly shaded). The number of parasitoids in locations 2 (Stelmachowo) and 4 (Falenty) (both intensely insolated) were comparable. The statistical analysis showed significant differences in abundance of horse chestnut leafminer parasitoids in the studied locations (F =3.57; df= 5.66; p Value = 0.0065) (Fig. 3).

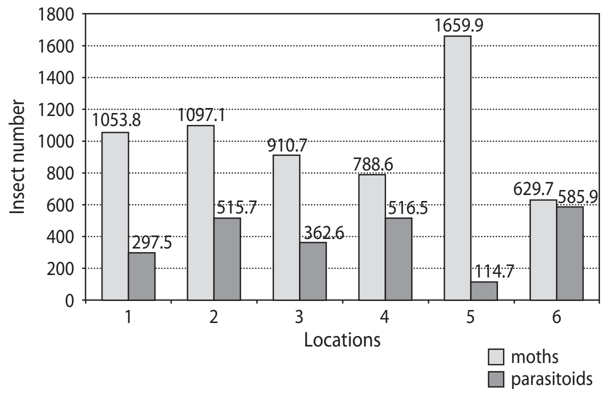

Fig. 2. The total number of moths and parasitoids of *C. ohridella* in the years 2004–2006 as assessed per 100 g of leaf dry mass

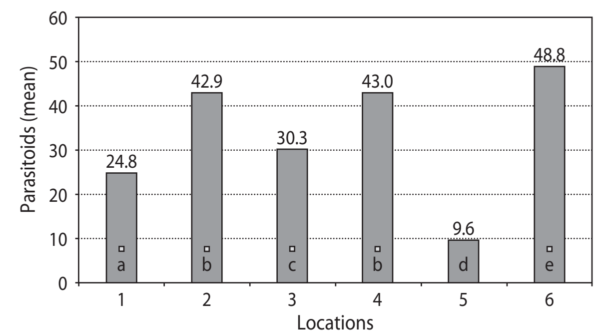

Fig. 3. Differences in parasitoid abundance in the years 2004–2006 as assessed per 100 g of leaf dry mass (letters denote homogenous groups)

Also, statistically significant differences were found in the numbers of *C. ohridella* moths (F = 2.02; df = 5.66; p-Value = 0.0878) (Fig. 4). The largest number of moths flew out from horse chestnut leaves collected in location 5 (greatly shaded), the lowest – in location 6 (intensely insolated). Two homogenous groups were identified, one for locations 3, 4, 6 and two for locations 1 and 2 (Fig. 4).

Minotetrastrichus frontalis (Nees) was the dominant species in all analysed groups and accounted for 70% of all parasitoids to be followed by *Pnigalio agraules* (Walker) (ca 25%) (Fig. 5). The share of other subdominant and accessory species in the assemblage

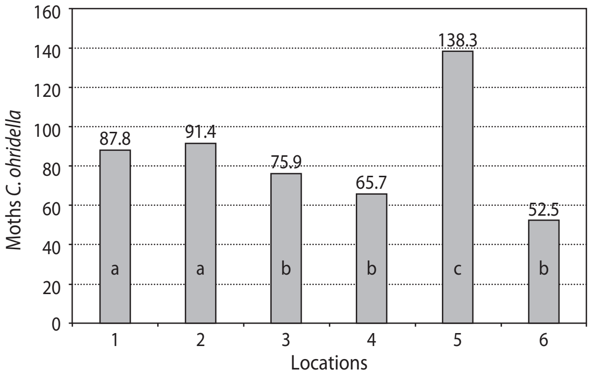

Fig. 4. Effect of the location on the abundance of moths of the horse chestnut leafminer in the years 2004–2006 as assessed per 100 g of leaf dry mass (letters denote homogenous groups)

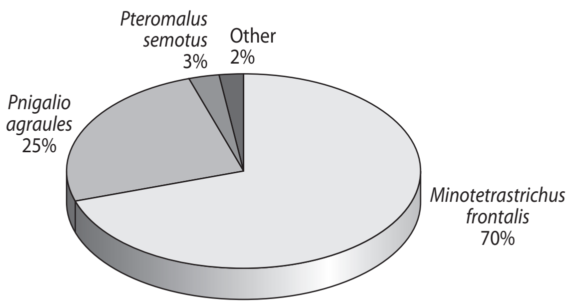

Fig. 5. Percentage share of parasitoids of the horse chestnut leafminer reared in the years 2004–2006

of parasitoids of the horse chestnut leafminer varied in successive years. *Pteromalus semotus* (Walker) (ca 3%) was considered subdominant and its presence was determined in all samples. The remaining parasitoid species identified during rearing were: *Cirrospilus pictus* (Nees), *Cirrospilus viticola* (Rondani), *Cirrospilus vittatus* Walker, *Cirrospilus elegantissimus* Westwood, *Cirrospilus talitzkii* Bouček, *Cirrospilus variegates* (Masi), *Pnigalio soemius* (Walker), *Pteromalus* sp., *Closterocerus trifasciatus* Westwood and two species from the family Ichneumonoidae: *Scambus annulatus* (Kiss) and *Itoplectis alternans* (Gravenhorst). Abundance of the above specified species ranged from 2% to a fraction of percent depending on the location and study year (Fig. 6).

The highest level of parasitism of the horse chestnut leafminer was found in location 6 and was 48.8%, while the lowest was in location 5–9.6% (Fig. 7). The parasitism level in remaining locations approximated to 30%.

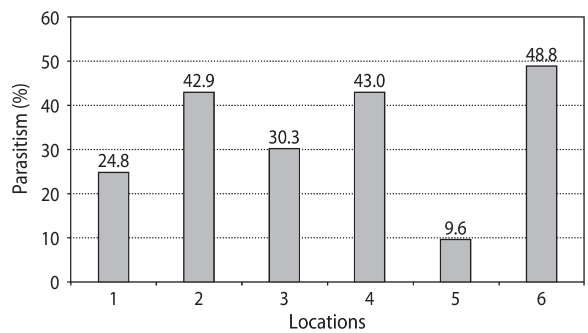

Fig. 7. Level of parasitism of the horse chestnut leafminer in analysed locations in the years 2004–2006 (WPS method)

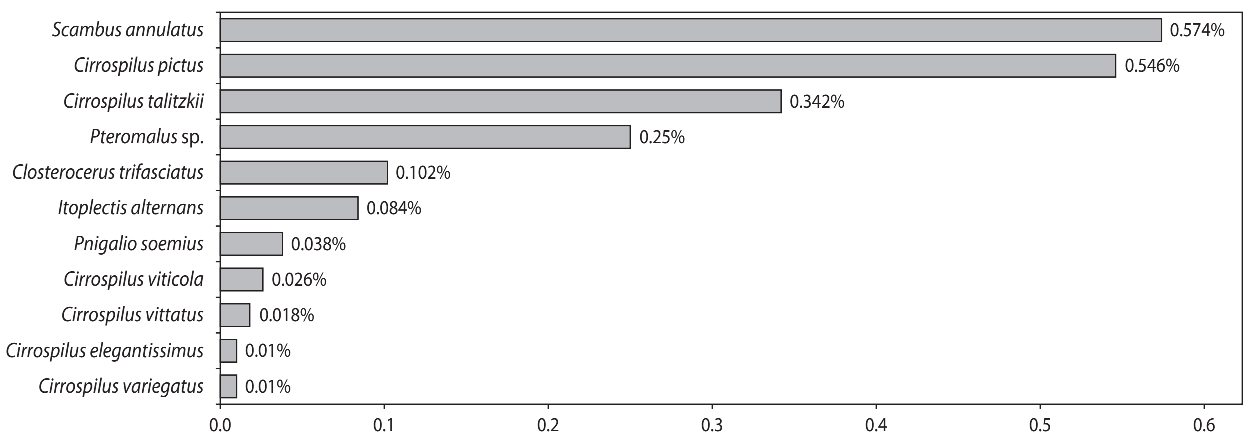

Fig. 6. Percentage share of accessory species in the assemblage of parasitoids of the horse chestnut leafminer reared in the years 2004–2006

Discussion

The horse chestnut (*A. hippocastanum*) has been planted in Poland as one of the first tree species of foreign origin since the 17th century, mainly in parks or along avenues. The appearance and mass occurrence of the horse chestnut leafminer (*C. ohridella*) has had a negative effect on decorativeness of the attacked trees The curiously low activity of horse chestnut leafminer's parasitoids was the reason behind numerous studies on the level of parasitism of phytophage population in different European countries. The results confirmed that the level of parasitism varies and, in general, does not exceed 20% of pest population.

This study carried out in 2004–2006 confirmed that the horse chestnut leafminer developing in insolated crowns of trees planted as roadside tree avenues or as single trees in parks is more often attacked by parasitoids. A very small number of parasitoids was found in forested locations, where sunray penetration was weaker compared to the trees subject to this experiment.

The presence and diversification of the surrounding vegetation had no effect on the number of parasitoids and their species diversity. This indicates that most of the parasitoids attacking *C. ohridella* do not derive from other host insect species (Girardoz et al. 2006; Grabenweger 2004). Similar results are quoted by Jäckel et al. (2006), who observed a high level of parasitism of horse chestnut leafminers on insolated tree avenues in the city centre in comparison with a much lower degree in "natural "out-of-town locations. Insolation of tree crowns and higher temperatures most likely stimulate females to lay eggs or accelerate individual development stages of parasitoids. Faster development of parasitoids due to higher temperatures, during the same time-unit, should result in a higher level of parasitism of the populations developing in insolated locations thanks to a, hypothetically, larger number of females depositing eggs in places characterized by a shorter average time of parasitoid development. Further studies are needed on both parasitoid behaviour associated with egg deposition as well as its development on the host to answer the question: how does the level of parasitism of a given population increase in insolated locations?

The confirmation of a correlation between the level of parasitism and insolation of location sheds a new light on the possibilities of estimating individual populations of the horse chestnut leafminer. It seems that this factor should be taken into consideration while planning experiments on comparing the level of parasitism of different populations. Moreover, this dependence may to some extent explain the high variability of the results obtained in earlier studies (Boisneau et al. 2004; Grabenweger and Lethmayer 1999; Freise and Heitland 2001; Freise et al. 2002; Stolz since 1997; Stojanović and Marković 2004; Volter 2004).

The complex of parasitoids of the horse chestnut leafminer was similar for all locations under the study. Several rare species (*Cirrospilus elegantissimus*, *Cirrospilus talitzkii*, *Cirrospilus variegatus*, *Pnigalio soemius*, *Pteromalus* sp., *Closterocerus trifasciatus*) were only found on intensely insolated sites. The very poor parasitoid species composition was associated with the shaded trees. The observed species composition was identical to those quoted in the research results from other Europe's regions (Grabenweger and Lethmayer 1999; Hellrigl 2001; Freise et al. 2002; Grabenweger 2003; Girardoz et al. 2006).

M. frontalis, the dominant species in all locations, played the leading role also in the studies by other researchers (Grabenweger 2003; Girardoz et al. 2006). This is, however, a gregarious species (it lays many eggs in the host's flesh) which can explain why it outnumbers other species in all samples. The second dominant species, *P. agraules,* was also found frequent in other authors' experiments (Girardoz et al. 2006).

The level of parasitism of the horse chestnut leafminer in many locations in Europe varies from 3% to 21% (Grabenweger 2003; Grabenweger et al. 2005, Girardoz et al. 2006). It is definitely lower than the level of parasitism of other leafmining species where damage often exceeds 50% (Askew and Shaw 1979; Gibogini et al. 1996; Maier 1984). The results are different due to, among others, application of various methods of determining the parasitism degree. Grabenweger and Lethmayer (1999) propose to assess the level of parasitism of horse chestnut leafminers as a ratio of their larvae and pupae to the number of reared parasitoids. In the present study, the assessment method is modified: the reared moths of the horse chestnut leafminer are counted instead of its larvae and pupae.

In the present experiments, the level of parasitism of the hibernating population was studied, as the composition and number of parasitoid species at the end of the growing season. As horse chestnut leafminer moths do not hatch unless winter diapause is broken, the taken samples were exposed to low temperatures. This method may leave out the mortality of the horse chestnut leafminer caused by fungi, bacteria or viruses, however these factors have a very limited effect on the total number of the examined phytophages.

Some authors suggest that the main reason behind the low number of parasitoids of the horse chestnut leafminer is the lack of springtime synchronization of insect appearance with the development of host leaves (Grabenweger and Lethmayer 1999; Grabenweger 2004). However, it seems that there can be additionally a certain mechanisms in Eulaphidae preventing egg deposition on one leaf. The presence of "pheromone markers" indicating penetration of the spot by other females may prevent facultative parasitism observed in the dominant parasitoids of the horse chestnut leafminer. Such a biological mechanism based on chemical markers could, to a certain degree, explain the low level of parasitism of the horse chestnut leafminer population, where the number of mines on one leaf blade may reach several dozen. Therefore, it seems that adaptation of parasitoids to a new host, *C. ohridella,* may be a lengthy process.

Conclusions

– The sun-exposure of *A. hippocastanum* influenced the assemblages of *C. ohridella* parasitoids. The highest number of parasitoids was observed on intensely insolated trees, and the lowest on greatly shaded ones.
– *M. frontalis and P. agraules* were the dominant species in the assemblage of parasitoids of the horse chestnut leafminer and accounted for about 90% of the total number of individuals reared during the study period.
– The percentage of parasitism of *C. ohridella* ranged from 9% to 50%. The highest level of parasitism was confirmed in the Obory location (intensely insolated), while the lowest – in the location: Protected Stand "Modrzewina" (greatly shaded). In the remaining locations observed paratistism ranged from 20% to 40%.

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