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ERYSIPHE FLEXUOSA (FUNGI, ERYSIPHALES) – LIFE STRATEGIES AND THREATS TO CHESTNUT TREES INCLUDING *CAMERARIA OHRIDELLA* (LEPIDOPTERA, GRACILLARIIDAE) PEST IN THE URBAN ENVIRONMENT

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Key words: *Erysiphe flexuosa*, powdery mildew, *Aesculus spp.*, *Cameraria ohridella,* host infestation.

Abstract

This study was aimed at analyzing the occurrence and at monitoring the developmental cycle of *Erysiphe flexuosa* (Peck) U. Braun & S. Takamatsu (syn. *Uncinula flexuosa*) on leaves of chestnut tree in the urban environment in 2013–2014, as affected by the presence of horse-chestnut leaf miner *Cameraria ohridella* Deschka & Dimic. A high occurrence of both these species and high infestation of plants were noted in each study year. Powdery mildew was infesting both *Aesculus hippocastanum* L. and *A. x carnea* Hayne, with a clear preference for the second host, whereas *C. ohridella* occurred mainly on *A. hippocastanum*. The highest number of recorded cases of species presence was found in the samples wherein these organisms cooccurred. It was demonstrated that *E. flexuosa* underwent a complete developmental cycle and accomplished its life strategies in the presence of *C. ohridella*, however pest presence was found to affect the disease index and the number of chasmothecia developed by *E. flexuosa*.

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ERYSIPHE FLEXUOSA – STRATEGIE ŻYCIOWE I ZAGROŻENIE DLA KASZTANOWCÓW Z UWZGLĘDNIENIEM SZKODNIKA *CAMERARIA OHRIDELLA*

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Słowa kluczowe: *Erysiphe flexuosa*, mączniaki prawdziwe, *Aesculus spp.*, *Cameraria ohridella,* stopień porażenia.

Abstract

Celem pracy była analiza występowania oraz prześledzenie cyklu rozwojowego *Erysiphe flexuosa* (Peck) U. Braun & S. Takamatsu na liściach kasztanowca w środowisku miejskim w latach 2013–2014, z uwzględnieniem obecności szrotówka *Cameraria ohridella* Deschka & Dimic. W każdym roku badań notowano wysoki udział tych gatunków i wysokie porażenie roślin. Mączniak prawdziwy atakował zarówno *Aesculus hippocastanum* L., jak i *A. x carnea* Hayne, z wyraźną preferencją drugiego żywiciela, natomiast *C. ohridella* wystąpił głównie na *A. hippocastanum*. Najwięcej notowań dotyczyło prób, w których organizmy te występowały razem. Stwierdzono, że *E. flexuosa* przechodzi pełny cykl rozwojowy i realizuje w pełni swoje strategie życiowe w obecności *C. ohridella*. Jednak obecność owada *C. ohridella* wpływa na indeks chorobowy oraz liczbę owocników tworzonych przez *E. flexuosa*.

Introduction

Powdery mildew of a chestnut tree – *Erysiphe flexuosa* (Fungi, Erysiphales) is an obligatory parasite developing on plants from the genus *Aesculus*. Symptoms of infestation with this pathogen were described on various species of chestnut trees: *A. arguta* Buckl., *A. georgiana* Sarg., *A. glabra* Willd, *A. hippocastanum* L., *A. pavia* L., and *A. x carnea* Hayne. *E. flexuosa* occurs in the North America (Glawe and Dugan 2006), it was also described in Eastern Asia (Braun 1987) and contemporarily also in Europe, where it had been brought into at the end of the XXth century. This pathogen turned out to be a highly invasive species, fast spreading in many countries (Ale-Agha et al. 2000, Bolay 2000, Denchev 2008, Ing, Spooner 2002, Nali 2006 Zimmermannova-Pastirčáková and Pastirčák 2002, Kiss et al. 2004, Milevoj 2004, Stankeviciene et al. 2010, Tozlu and Demirci 2010, Talgo et al. 2012). In Poland, first communications concerning the occurrence of *E. flexuosa* have appeared in the year 2002 (Adamska 2002, Piątek 2002). So far, this species has been

recorded in Poland on *A. hippocastanum, A pavia*, *A.* × *carnea* in such cities as: Szczecin (ADAMSKA 2002), Tarnów (PIĄTEK 2002), Lublin (WOŁCZAŃSKA and MUŁENKO 2002), Poznań, Inowrocław, Ciechocinek (WERNER 2007, WERNER et al 2009, KAROLEWSKI et al. 2010), and Olsztyn (SUCHARZEWSKA et al. 2011, 2012). The aforementioned works provide important information not only about the occurrence of *E. flexuosa* but also about its morphological traits, about the presence of a powdery mildew hyperparasite *Ampelomyces quisqualis* Ces., and about the susceptibility of different species of chestnut tree to this parasite.

Apart from powdery mildew, chestnut trees may also be infested by *Guignardia aesculi* (Peck) V.B. Stewart, a fungus inducing the chestnut leaf blotch as well as by a butterfly *Cameraria ohridella* Deschka & Dimic. Considering that the chestnut tree constitutes a permanent and important element of the Polish dendroflora (SENETA 1994), especially much attention has been paid to *C. ohridella* pest being very harmful to chestnut trees, the presence of which in Poland was first documented in the year 1998 (ŁABANOWSKI and SOIKA 1998). This insect feeds on leaves, which leads to damage of the assimilation tissue and, consequently, to tissue death (BARANOWSKI et al. 2002). Fungi and pests may simultaneously colonize the same surface of a leaf and differently act on one another. For instance, it has been demonstrated that the presence of chemical compounds, produced during mycotic infections, affected a reduction in the number of eggs laid by horse-chestnut leaf miner, and that the possibility of mutualistic interactions between these organisms is small (JOHNE et al. 2006, 2008).

In Poland, investigations addressing the issues linked with *Erysiphe flexuosa* parasite and *Cameraria ohridella* pest are mainly focused on their occurrence, evaluation of their harmfulness to a host plant expressed by the diseases index, and on determination of the susceptibility of a chestnut species or variety to infestation (WERNER et al. 2002, DZIĘGIELEWSKA et al. 2005). In turn, little information is available regarding the biology of *E. flexuosa* development in the presence of *C. ohridella*.

The objective of this study was to analyze the life cycle and vital strategies of *Erysiphe flexuosa* on chestnut trees occurring in the urban environment, considering *Cameraria ohridella* as a biotic factor significant for the condition of a host plant.

Material and Methods

Determination of the extent of leaves infestation with *Erysiphe flexuosa* and leaves damage by *Cameraria ohridella*

The study was carried out on the area of the city of Olsztyn on *Aesculus hippocastanum* and *A.* x *carnea* in two vegetative seasons: 2013 (55 stations), 2014 (64 stations). The analyzed trees, at different age, were growing in various parts of the city (communication tracts, parks, neighborhoods). In 2013, samples were collected once – in September, whereas in 2014 the material was collected four times at the beginning of the following months: June, July, August, and September, in order to analyze the developmental cycle of powdery mildew within the entire vegetative season. One sample included 10 leaves collected randomly from the bottom part of a head of each tree.

The following research tasks were planned:

1. Determination of the extent of leaves infestation with *Erysiphe flexuosa* and leaves damage by *Cameraria ohridella.*
2. Analysis of the developmental cycle of *Erysiphe flexuosa* considering the presence of *Cameraria ohridella.*
3. Determination of the odds ratio OR.

The extent of host plant infestation with *Erysiphe flexuosa* was calculated for each sample according to the Mc Kinney's formula (Dynowska 1994):

$$R = \frac{\Sigma(a \cdot b) \cdot 100\%}{N \cdot 4}$$

R – disease index expressed in per cents (index)

$\sum (a \cdot b) \cdot 100\%$ – sum of products obtained by multiplying the number of analyzed organs of plants (a) by the infestation degree (b)

N – total number of analyzed leaves (leaves)

4 – the highest degree of infestation in a five-point scale (from 0 to 4):

- 0 – no infestation;
- 1 – infestation of up to 10% of leaf surface;
- 2 – infestation of 11–25% of leaf surface;
- 3 – infestation of 26–50% of leaf surface;
- 4 – infestation of 51–100% of leaf surface.

Ultimately, the R value considered in the analysis of results was computed based on the arithmetic mean and described as a mean degree of infestation.

In addition, the extent of leaf damage by *Cameraria ohridella* was evaluated for each sample according to a five-point scale adopted by Baranowski et al. (2002):

– 0 – no damage;
– 1 – weak damage (up to 10% of damaged leaf surface);
– 2 – medium damage (10–25%);
– 3 – severe damage (25% to 70%);
– 4 – very severe damage (70%).

In the Results section, arithmetic mean was provided for each sample and described as the mean degree of infestation/ mean degree damage.

Infestation degree and damage degree vs. age of host plant. Analysis of the developmental cycle of *Erysiphe flexuosa* considering the presence of *Cameraria ohridella*

The average degree of host plant infestation with *E. flexuosa* and the average degree of host plant damage by *C. ohridella*. The age of the analyzed tress was determined based on a table developed by MAJDECKI (1980–1986).

An SZX9 stereoscopic microscope (Olympus) was used to determine:

a) developmental stage of *E. flexuosa* (anamorph, teleomorph);

b) number of chasmothecium-type fruiting bodies, mature and immature, per 1 cm^2 of the surface area of each infested leaf in the sample; the number of chasmothecia presented in the Results section was computed based on an arithmetic mean;

c) BX41 optical microscope (Olympus) was used to analyze 10 randomly chosen, morphologically mature chasmothecia; a 3° scale, adopted in a previous own study (Sucharzewska 2009), was applied to evaluate:

– degree of development of appendages: 0 – chasmothecia without appendages, I – chasmothecia with not fully developed appendages, and II – chasmothecia with fully developed appendages,

– degree of maturity of chasmothecia: 0 – chasmothecia without sacks and ascospores, I – chasmothecia with sacks but without formed ascospores, and II – chasmothecia with sacks and ascospores.

Determination of the odds ratio OR in order to:

– compare the degree of plant infestation with *Erysiphe flexuosa* in the samples with and without horse-chestnut leaf miner. The odds ratio indicates the ratio of the likelihood of severe infestation (> 60%) in group *A* (without horse-chestnut leaf miner) to the likelihood of low infestation in group *B* (with horse-chestnut leaf miner),

– the number of chasmothecia of *E. flexuosa* in the samples with and without horse-chestnut leaf miner. The odds ratio indicates that ratio of the likelihood producing chasmothecia in group *A* (without horse-chestnut leaf miner) to the likelihood of producing chasmothecia in group *B* (with horse-chestnut leaf miner).

Results

In total, there were analyzed 311 samples (3110 leaves) collected from *A. hippocastanum* (246 samples) and *A. x carnea* (45 samples). The presence of both *Erysiphe flexuosa* and *Cameraria ohridella* was noted on chestnut tree leaves in each study year. The percentage of the samples infested with the fungus only reached 13%, whereas the percentage of the samples invaded by the pest only reached 26%. In turn, samples colonized by these two organisms together constituted as much as 59% of all samples (Figure 1). The parasite and the pest showed preferences for different species of chestnut tree: *E. flexuosa* attacked both *A. hippocastanum* and *A. x carnea* with preference for *Aesculus x carnea*, whereas *C. ohridella* was more frequently foraging leaves of *A. hippoccastanum* (Table 1).

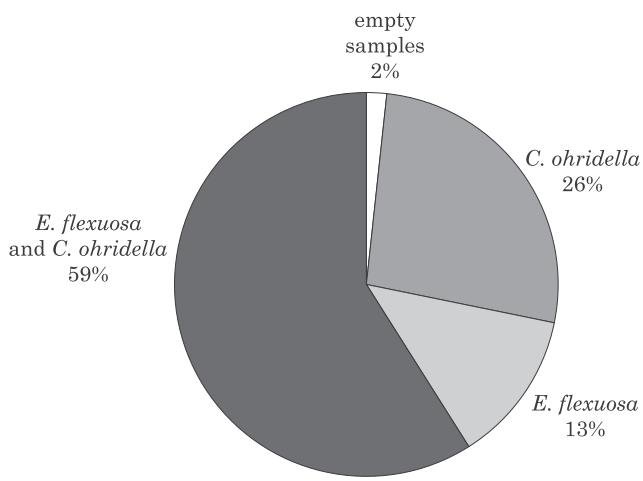

Fig. 1. The percentage contribution of samples with the analyzed species *Erysiphe flexuosa* and *Cameraria ohridella* in the study period (2013, 2014)

Table 1

The percentage contribution of *A. hippocastanum* and *A.* × *carnea* infested by *E. flexuosa* and *Cameraria ohridella* in the study years (2013, 2014)

Plant	2013		2014	
	E. flexuosa	*C. ohridella*	*E. flexuosa*	*C. ohridella*
A. hippocastanum	47%	82%	60%	76%
A. x carnea	100%	11%	100%	22%

When analyzing the mean infestation/damage degree from September of 2013 and 2014, it was found that the plants were to a greater extent damaged by *C. ohridella* – 62% and 73% respectively, whereas in the case

of *E. flexuosa* the disease index reached 28% and 31%, respectively (Figure 2). The study demonstrated the effect of insect presence on the degree of plants infestation by the powdery mildew. If only *E. flexuosa* was noted in the samples, the mean degree of infestation was two times higher than in the samples invaded by both *E. flexuosa* and *C. ohridella* (Figure 3). The odds ratio reached 23.59 for the compared groups. It means that in group A the likelihood of severe plant infestation by *E. flexuosa* was almost twenty four times higher than in the samples with co-occurrence of the fungus and the insect (group *B*).

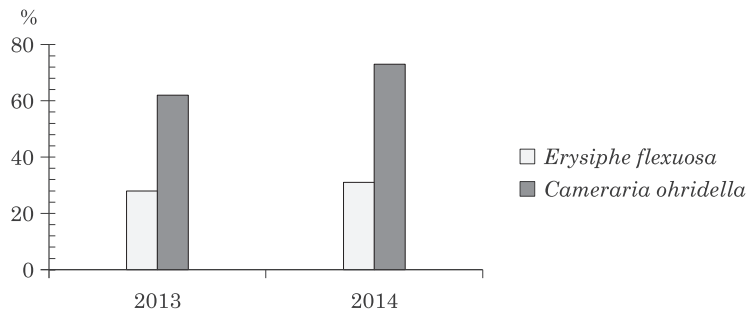

Fig. 2. Mean infestation degree [%] of host plants by *Erysiphe flexuosa* and *Cameraria ohridella* in the study period (2013, 2014)

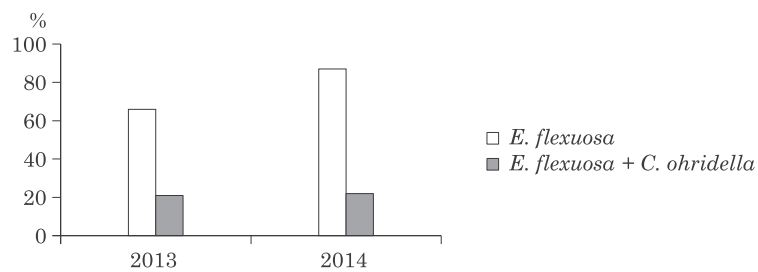

Fig. 3. The effect of the presence of horse-chestnut leaf miner *Cameraria ohridella* on the mean degree of plants infestation by *Erysiphe flexuosa* in study years (2013, 2014)

The age of chestnut trees was demonstrated to affect differences in the degree of their infestation/damage. The powdery mildew caused the strongest infestation of young trees up to 10 years, whereas the insect – the strongest damaged of the oldest trees (Figure 4).

The presence of *E. flexuosa* was noted already at the beginning of June. The percentage of positive samples in this month reached 21% and was successively increasing in the subsequent months – to 78% in September, with the simultaneous, stable and high contribution of *C. ohridella* reaching over 80% (Figure 5).

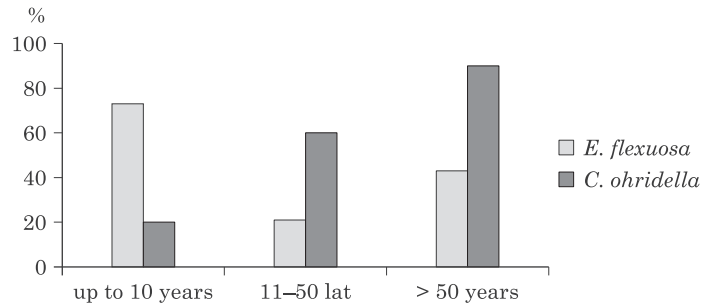

Fig. 4. The effect of tree age on the mean degree of samples infestation by *Erysiphe flexuosa* and *Cameraria ohridella*

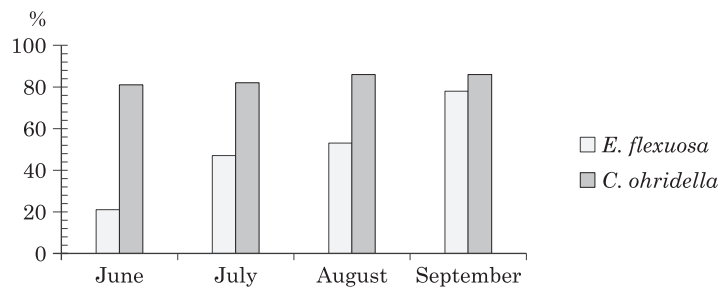

Fig. 5. Percentage of contribution of samples with *Erysiphe flexuosa* and *Cameraria ohridella* in particulars months in 2014

Fungus *Erysiphe flexuosa* was observed to undergo a complete developmental cycle. Its anamorphous and teleomorphous stages were noted in each studied month, also in the samples infested by the pest. Chasmothecia of the parasite were appearing already in June and were produced until the end of the vegetative season, mainly at the bottom part of leaf. The chasmothecia were always at a different developmental stage – from young ones (white, yellow and orange) to mature ones (brown, dark-brown or black). The mature chasmothecia were prevailing through the entire study period (Figure 6). The highest number of chasmothecia was produced in August and September. Differences were observed in the mean number of produced chasmothecia in the samples without horse-chestnut leaf miner (group *A*) compared to the samples attacked by the insect (group *B*). In the samples with co-occurrence of both organisms, the mean number of *E. flexuosa* chasmothecia was lower (Figure 7). The odds ratio calculated for the compared groups reached 1.82, which means that in group A the chance for producing chasmothecia was less than twofold higher than in the samples attacked simultaneously by the fungus and by the insect (group *B*). The highest number of chasmothecia (497/cm^2) was determined on *A. x carnea* in the sample with 100% infestation, without the presence of *C. ohridella*.

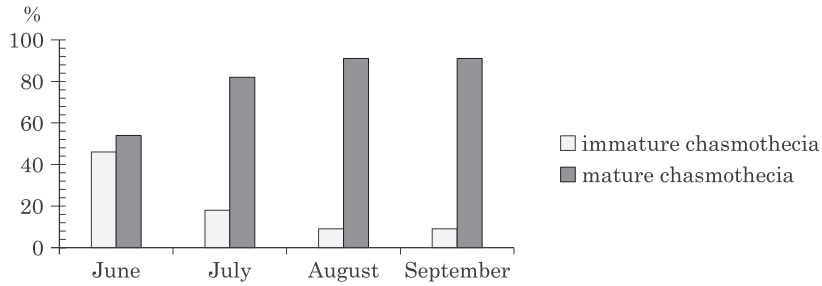

Fig. 6. Mean percentage of *Erysiphe flexuosa* chasmothecia per 1 cm^2 of the surface of infested leaves in particular months in 2014

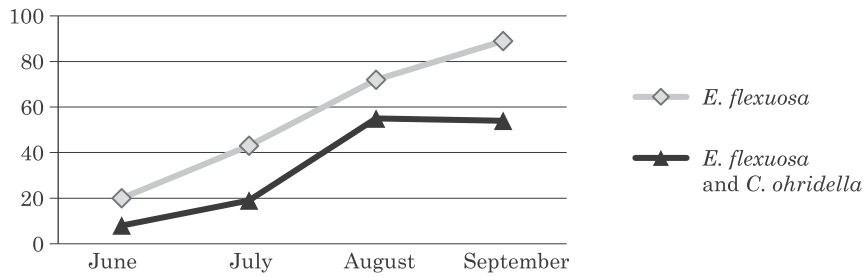

Fig. 7. The mean number of chasmothecia of *Erysiphe flexuosa* with and without *Cameraria ohridella*

Throughout the vegetative season, chasmothecia of *E. flexuosa* had appendages being at various developmental stages, however already in July most of the chasmothecia had fully developed appendages at stage II of development (Figure 8). The process of formation and development

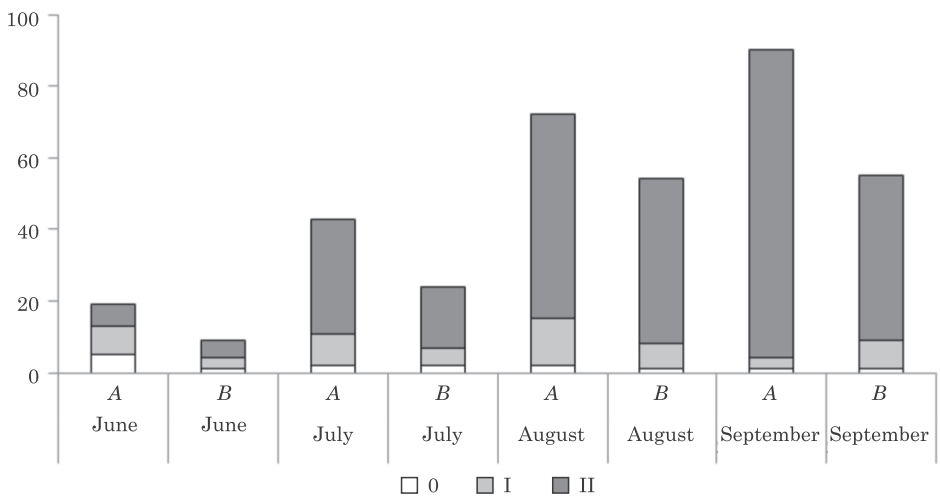

Fig. 8. Mean number of mature chasmothecia of *Erysiphe flexuosa* with appendages in various developmental stages in particular months of the study in the samples infested by the fungus alone (*A*) and in the samples co-infested by the fungus and by the insect (*B*) in 2014 year

of chasmothecia was not impaired by the presence of *C. ohridella*. Asci with developed ascospores were noted already in June. In each studied month, the highest number was determined for the chasmothecia at the II stage of development. Empty chasmothecia, without developed asci nor and spores, constituted 7%.

Discussion

The most important element of the life strategy of parasites, whose diaspores are present in the entire biosphere, is to meet an appropriate host that would ensure their survivability in various environmental conditions (MUŁENKO 1998). An urbicenosis is a type of habitat wherein many species of parasitic fungi fully accomplish their vital roles (BERNADOVIČOVÁ and IVANOVÁ 2008, DYNOWSKA and SUCHARZEWSKA 2005, HOŁOWNIA and KOSTRZEWSKA 1991, JARVIS et al. 2002, SUCHARZEWSKA and DYNOWSKA 2005, SUDNIK-WÓJCIKOWSKA 1998, SUCHARZEWSKA 2009, 2010). It was also confirmed in the presented study, wherein *Erysiphe flexuosa* occurred on chestnut trees in the urban environment in each year of the study. The high prevalence of powdery mildew and a high infestation degree of host plants are indicative of the invasive character of this species. It confirms earlier findings reported by: DZIĘGIELEWSKA et al. (2005), STANKEVICIENE et al. (2010), WERNER et al. (2009, 2012), WOŁCZAŃSKA and MUŁENKO (2002) or KAROLEWSKI et al. (2010). The spreading of powdery mildews results from, i.a., their capability to infect plants in a wide range of temperature and humidity. In most species, the germination of spores may occur even at the minimal relative air humidity, owing to the sufficient water content in the cell. For these reasons, they easily extend their geographical range (BRAUN 1995). An important strategy of parasites is their high reproductive capability, expressed by the formation of structures enabling their spreading, and their ability to survive in unfavorable environmental conditions. In the case of powdery mildews, these roles are played by conidiospores and chasmothecia (BRAUN 1987). Their production throughout the vegetative season allows the parasites accomplishing these strategies. In the reported study, we observed a complete developmental cycle of *E. flexuosa* – the fungus was producing both conidiospores and chasmothecia. According to literature data, powdery mildews undergo the anamorphous stage at the early phase of development, whereas they enter into the teleomorphous stage usually in the autumn period, i.e. at the end of the developmental cycle (SAŁATA 1985). In turn, in our study, the chasmothecia of *E. flexuosa* were beginning to

appear already in June and were produced throughout the vegetative cycle. A similar observation was made for *Erysiphe palczewskii* infesting *Caragana arborescens* (SUCHARZEWSKA and DYNOWSKA 2005), also an extrinsic and invasive species of powdery mildew (MUŁENKO et al. 2010). The tasks of chasmothecia include both producing ascospores as a source of infection as well as surviving winter. The strategy of potentiating and improving reproduction is an important trait of parasites. The production of chasmothecia by *E. flexuosa* over the entire vegetative season is indicative of the vast reproductive potential of this species and perhaps, it provides secondary infections and a high likelihood of surviving unfavorable conditions. It emphasizes the invasive character of this parasite, which from its appearance in Europe has been displaying the capability of undergoing the reproductive stage (ALE-AGHA et al. 2000, PIĄTEK 2002). In most cases, the extrinsic species spreading over new areas are noted at the asexual stage, for example *Erysiphe deutziae* (Bunkina) U. Braun & S. Takam or *Erysiphe russellii* (Clinton) Braun & Takam (BOLAY et al. 2005). In the case of the first, the sexual stage has never been noted, whereas in the case of the latter – already ca. 80 years after its first record (GELJUTA and MARCHENKO 1987). Chestnut trees are host plants also to *Cameraria ohridella*. The reported study confirmed the presence of this dangerous insect. Its percentage contribution in the samples and the degree of plant damage were significantly higher compared to powdery mildew. The overlapping of ecological niches of parasites makes some interactions between organisms likely. These may include competing for habitat and feeding areas or entering into synergistic reactions that result in the establishment of a new system, the so-called "disease complex". Such a phenomenon was observed in the case of the Dutch elm disease, induced by *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* fungi as well as by *Scolytus* spp. and *Hylurgopinus rufipes* bark beetles (after JOHNE et al. 2008). In the case of *E. flexuosa* and *C. ohridella*, works of German scientists demonstrated chemical-and-ecological predispositions for antagonistic interactions during co-colonization of a host plant (JOHNE et al. 2006, 2008). These investigations showed the capability of *C. ohridella* to detect volatile chemical compounds synthesized by fungi (1-Octen-3-ol and 3-octanone) and produced by plants (5-ethyl-2 (5H) –furanone) in response to the microbiological attack, which results in a reduced number of eggs laid by *C. ohridella*. Perhaps, this factor which minimizes the occurrence of horse-chestnut leaf miner on leaves also ensures *E. flexuosa* the possibility of colonizing a healthy, undamaged plant tissue and realizing its vital functions. It could be confirmed by, demonstrated in this study, significantly higher number of the samples with co-occurrence of these organisms (59%) and

production of well-developed chasmothecia with asci and spores by *E. flexuosa*. It is feasible due to the fact that both organisms attacking chestnut trees were noted since the beginning of June, which suggests concomitant development of these parasites at the beginning of the vegetative season. The presence of the analyzed pathogenic factors was also reported in other works (DZIĘGIELEWSKA et al. 2005, JOHNE et al. 2008, WERNER 2007, WERNER et al. 2012). Results of this study and findings of other authors (DZIĘGIELEWSKA et al. 2005, KUKUŁA-MŁYNARCZYK and HUREJ 2007, WERNER et al. 2012) show some preferences of both parasites for infesting different species of chestnut trees. The damage degree of red chestnut trees by *C. ohridella* is very low, whereas *E. flexuosa* attacks both white and red chestnut trees, with a very high occurrence on *Aesculus* x *carnea* (100% infestation in all samples). Such a high degree of plant infestation by this fungus, without the presence of the insect, may be due to both host susceptibility and high availability of a feeding base which is limited when the insect feeds on the same plant. It is indicated by the calculated odds ratio (OR), according to which the degree of leaves infestation by *E. flexuosa* would be significantly higher without the presence of *C. ohridella*.

Conclusions

Results achieved in this study enable concluding that the *Erysiphe flexuosa* fungus fully accomplishes its life strategies in the urban environment. Despite insect's presence *Cameraria ohridella*, the fungus has the possibility of developing and producing a high number of chasmothecia with spores, which – as a result of the sexual process – ensure the parasite the possibility of adjusting to variable environmental conditions that are especially dynamically changing in the urban environment. Therefore, investigations focused mainly on eradication of horse-chestnut leaf miner (BARANOWSKI and DANKOWSKA 2012, KROPCZYŃSKI et al. 2006, ŁABANOWSKI et al. 2008), should also take into account the presence of *E. flexuosa* which poses great threat to chestnut trees considering its invasive character.

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