OCCURRENCE OF THE FUNGI FROM THE GENUS *AMPELOMYCES* – HYPERPARASITES OF POWDERY MILDEWS (ERYSIPHALES) INFESTING TREES AND BUSHES IN THE MUNICIPAL ENVIRONMENT

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ABSTRACT

The studies refer to the phenomenon of hyperparasitism in the municipal environment. The paper presents the occurrence of fungi of the genus *Ampelomyces* on Erysiphales – important group of phytopathogenic fungi. For the first time in Poland analyzed degree of infestation of Erysiphales mycelium by *Ampelomyces* and effect of the hyperparsites on the degree of infestation plants by Erysiphales. The high participation of the *Ampelomyces* was noted in each year of the study. Substantial differences were noted in the occurrence of *Ampelomyces* depending on the developmental stage of the host fungi and considerable differences in the prevalence of the hyperparasites on particular Erysiphales species. In all cases examined, the mean index of infestation of host plants by Erysiphales was higher than the mean degree of infestation of powdery mildew mycelium by *Ampelomyces*. The results indicate that under natural conditions they do not play any significant role in the reduction of the degree of infestation of host plants by Erysiphales and do not disturb drastically their life cycle.

KEY WORDS: *Ampelomyces*, Erysiphales, hyperparasites, municipal environment.

INTRODUCTION

Fungi from the genus *Ampelomyces* (Ces. ex Schlecht.) are hyperparasites colonizing mycelium, conidial spores and young fruiting bodies of powdery mildews (Erysiphales) – obligate patogens of plants (Kiss et al. 2004). *Ampelomyces* are widespread worldwide, they are thermophilic organisms, adapting well to various climatic conditions (Sundheim 1982). They belong to the class of Coelomycetes, Anamorfic fungi – formerly Deuteromycota (Sutton 1980; Kirk et al. 2001).

In literature there have been described ca. 46 species of the genus *Ampelomyces*, infesting different species of powdery mildew. The most up to day genetic researches of the ribosomal DNA (rDNA) of the ITS region confirm the existence of more that one species of the genus *Ampelomyces* (Kiss 1997; Kiss, Nakasone 1998; Kiss, Vajna 1995; Liang et al. 2007; Szentivànyi et al. 2005). In view of those investigations, the application of dual nomenclature – *Ampelomyces quisqualis* – for the description of fungi of that genus seems to be incorrect (Kiss et al. 2004).

Investigations referring to *Ampelomyces* are mainly focused on their utilization in a biological fight against powdery mildews that induce diseases of economically-important plants (Falk et al. 1995; Gu 1998; Kiss 2003; Kiss at al. 2004). In turn, still little attention is paid to the ecology of those parasites, their occurrence and effect on the development of Erysiphales in the natural environment (Kiss et al. 2004).

In Poland the *Ampelomyces* have been noted onto 44 species of Erysiphales belonging to six genera: *Erysiphe*, *Microsphaera*, *Podosphaera*, *Sphaerotheca*, *Uncinula* and *Oidium* (Mułenko et al. 2008). Those works document only sporadic presence of that mycoparasites without assays of the infestation degree of host mycelium nor the infested morphological structures. Those investigations were usually conducted in plants communities, characterized by a low degree of anthropopression. In contrast, there are sparse works referring to the presence of *Ampelomyces* on Erysiphales in the municipal environment, which report on the high prevalence of that mycoparasites (Madej and Antoszczyczyn 1965; Sucharzewska and Dynowska 2002). In Polish literature also is lack of information about degree of infestation of powdery mildew by *Ampelomyces* and their effect on the development of powdery mildews.

The presented research was aimed at evaluating the prevalence of fungi of the genus *Ampelomyces* and at determining the extent of mycelium infestation by those mycoparasites of powdery mildew species, infesting trees and bushes in the municipal environment.

MATERIAL AND METHODS

The research was conducted on the area of the city of Olsztyn during two vegetative seasons of 2005/2006. The experimental material were leaves of trees and bushes infested with Erysiphales. Ten leaves collected at random from each infested host plant served as one sample.

1. Each sample was determined for the extent of infestation of the host by powdery mildews, using a five-degree scale according to Mc Kinney's formula (Dynowska 1994):

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

where:

R – an index of plants infestation by Erysiphales expressed in per cents (%);

Σ (a × b) × 100% – the sum of products obtained by multiplying the number of plant organs examined (a) by the degree of infestation (b);

N – the total number of examined plants (relatively of leaves or fruits);

4 – the highest degree of infestation in the five-degree scale (0 – lack of infestation; 1 – up to 10%; 2 – 11-25%; 3 – 26-50%; 4 – 51-100% of infestation).

Species of Erysiphales were determined by means of keys by Braun (1987) and Sałata (1985). Nomenclature was adopted after Braun and Takamatsu (2000) and Braun et al. (2003). The host plants were determined according to Rutkowski (1998) and Bugała (1991). Nomenclature was adopted after Mirek et al. (1995).

2. Analyses were conducted for the presence of the hyperparasites – *Ampelomyces* on the mycelium of powdery mildews and for its effect on the development of the fungi examined.

– each sample was determined for the extent of infestation of powdery mildews mycelium with *Ampelomyces*, using the Mc Kinney's formula with appropriate modifications:

$$R_{Ampelomyces} = \frac{\Sigma (c \times d) \times 100\%}{N \times 4}$$

where:

$\mathbf{R}_{Ampelomyces}$ · an index of Erysiphales mycelium infestation by *Ampelomyces* expressed in per cents (%);

Σ (c × d) × 100% – the sum of products obtained by multiplying the number of collected plant organs infested with powdery mildew (c) by a given degree of infestation (d);

N – the total number of organs infested with Erysiphales;

4 – the highest degree in the five-degree scale.

The final values of infestation indices (R), taken into account in the analysis of results, were calculated based on an arithmetic mean for each fungal species and described as a mean index of infestation.

RESULTS

Over the two-year study, a total of 305 samples with powdery mildews were collected, including 186 in the year 2005 and 119 in the year 2006. The presence of *Ampelomyces* was detected on 111 samples, which constitutes 36% of the samples examined (Table 1). Hyperparasites were noted in each year of the study. No difference was observed in the percentage contribution of the samples with *Ampelomyces* in particular analytical seasons (38% in 2005, 39% in 2006).

Over the entire experimental period, there were collected 14 species of fungi of the order Erysiphales at the sexual stage on 20 host plants as well as 12 species at the asexual stage (*Oidium*) on 14 host plants (Table 1).

Substantial differences were noted in the occurrence of *Ampelomyces* depending on the developmental stage of the host fungi. Mycoparasites were detected onto 4 out of 12 species of Erysiphales at the asexual stage (*Oidium*) – which constitutes 33% as well as onto 9 out of 14 species of powdery mildew being at the sexual stage, which constitutes 64% (Table 2).

In addition, observations made in the study enabled determining considerable differences in the prevalence of *Ampelomyces* on particular Erysiphales species at the sexual stage. The highest number of the samples with mycoparasites was noted in the case of *Erysiphe vanbruntiana var. sambuci-racemosae* (92% of the samples), *E. flexuosa* (83%), and *E. berberidis* (69%) – (Fig. 1). The high prevalence of *Ampelomyces* was also reported in the samples with *Phyllactinia fraxini* (56%) and *E. palczewskii* (55%). In contrast, the lowest prevalence of *Ampelomyces* was observed in the samples with *P. guttata* (7%). In turn, the *Ampelomyces* were not detected on five Erysiphales species at the sexual stage: *E. adunca*, *E. alphitoides*, *E. tortilis*, *E. trifolii* and *Sawadaea bicornis* (Fig. 1).

The analysis of an infestation degree of mycelium of Erysiphales at the sexual stage by *Ampelomyces*, demonstrated a high mean index of infestation in the case of two species of powdery mildew: *E. berberidis* on *Mahonia aquifolium* (83%) and on *Berberis vulgaris* (59%), and *E. vanbruntiana var. sambuci-racemosae* on *Sambucus racemosa* (56%) (Fig. 2). The lowest mean degree of powdery mildews mycelium infestation by *Ampelomyces* was reported for: *P. guttata* on *Betula pendula* (6%) and for *S. tulasnei* on *Acer ginnala* (3%) (Fig. 2).

A considerably lower degree of Erysiphales mycelium infestation by *Ampelomyces* was determined at the asexual stage. A high infestation index was noted only at the conidial stage (*Oidium*) on *A. platanoides* (51%), whereas in the other Erysiphales species being at the asexual stage, the infestation index accounted for <13%.

In all cases examined, the mean index of infestation of host plants by Erysiphales was higher than the mean degree of infestation of powdery mildew mycelium by *Ampelomyces* (Fig. 2).

DISCUSSION

Hyperparasitism is one of the mechanisms of an antagonistic action, in which one fungus absorbs nutrients from another parasitic fungus being its host (Jeffries 1995).

TABLE 1. The list of Erysiphales species and host plants. The number of samples with powdery mildew infected and uninfected by *Ampelomyces* in the study years.

Lp.	*Erysiphales* species	Develop mental stage (sexual, asexual)	Host plant	2005-2006	
				The number samples with Erysiphales	The number samples with *Ampelomyces*
1.	*Erysiphe adunca* (=*Uncinula adunca*) (Wallr. Ex Fr.) Lév.	*Sexual* (*teleomorphic stage*)	*Salix cinerea* L.	3	0
2.	*Erysiphe alphitoides* (=*Microsphaera alphitoides*) Griff. Et Maubl.		*Quercus robur* L.	21	0
3.	*Erysiphe berberidis* (=*Microsphaera berberidis*) (DC. Ex Mérat) Lév.		*Mahonia aquifolium* (Pursh) Nutt. *Berberis vulgaris* L. *Berberis thunbergii* DC.	2 31 3	**2** **23** 0
4.	*Erysiphe flexuosa* Peck.		*Aesculus* spp.	24	**20**
5.	*Erysiphe hypophylla* (=*Microsphaera hypophylla*) Nevod.		*Quercus robur*	20	**4**
6.	*Erysiphe palczewskii* (=*Microsphaera palczewskii*) Jacz.		*Caragana arborescens* Lam.	20	**11**
7.	*Erysiphe syringae* (=*Microsphaera syringae*) (Schw.) Magn.		*Syringa vulgaris* L.	19	**3**
8.	*Erysiphe tortilis* (Wallr.) ex Fr.		*Cornus sanguinea* L.	2	0
9.	*Erysiphe trifolii* Grev.		*Caragana arborescens* Lamk.	1	0
10.	*Erysiphe vanbruntiana* (=*Microsphaera vanbruntiana*) Gérard		*Sambucus racemosa* L.	26	**24**
11.	*Phyllactinia fraxini* (DC.) ex Homma		*Fraxinus excelsior* L.	9	**5**
12.	*Phyllactinia guttata* (Wallr. Ex Fr.) Lév.		*Betula pendula* Roth. *Corylus avellana* L. *Fagus sylvatica* L.	21 7 2	**2** 0 0
13.	*Sawadaea bicornis* (=*Uncinula bicornis*) (Wallr. Ex Fr.) Lév.		*Acer campestre* L. *Acer ginnala* Maxim. *Acer negundo* L.	2 1 5	0 0 0
14.	*Sawadaea tulasnei* (= *Uncinula tulasnei*) Fuck		*Acer ginnala* *Acer platanoides* L.	7 26	**1** **11**
1.	*Oidium* (*Sawadaea bicornis*)	*Asexual* (*anamorphic stage*)	*Acer campestre*	2	0
2.	*Oidium* (*S. tulasnei*)		*Acer ginnala* *Acer negundo* *Acer platanoides* *Acer platanoides* odm. 'Fassens Black'	7 4 8 1	**1** **1** **2** 0
3.	*Oidium* (*Erysiphe berberidis*)		*Berberis vulgaris*	2	0
4.	*Oidium* (*Phyllactinia guttata*)		*Betula pendula*	1	0
5.	*Oidium* (*E. deutziae*)		*Deutzia scabra* Thumb. odm. 'Plena'	5	0
6.	*Oidium* (*E. eonymi* (=*M. eonymi*)		*Euonymus europaeus*L.	3	0
7.	*Oidium* (*E. alphitoides*		*Quercus robur*	14	0
8.	*and E. hypophylla*)				
9.	*Oidium* (*Podosphaera pannosa*)		*Rosa spp.*	3	0
10.	*Oidium* (*E. adunca*)		*Salix cinerea*	1	0
11.	*Oidium* (*E. syringae*)		*Syringa vulgaris*	1	0
12.	*Oidium* (*E. hedwigii* (=*M. hedwigii*)		*Viburnum opulus* L.	1	**1**
				305	**111 (36%)**

There are known around forty species of fungi claimed to be natural antagonists of Erysiphales (Kiss 2003). One of the best known hyperparasites of powdery mildews are fungi of the genus *Ampelomyces.* Investigations focusing

TABLE 2. Percentage contribution of samples with powdery mildew infected by *Ampelomyces* in different stage of development.

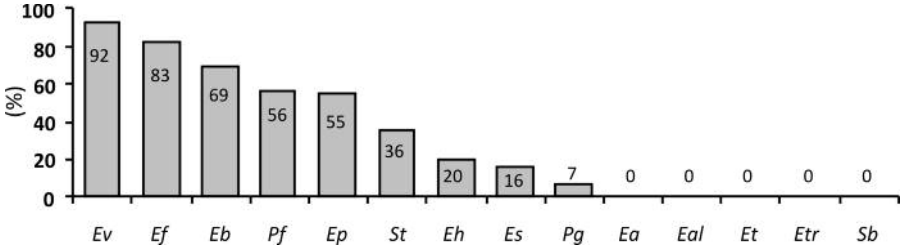
Developmental stage	Samples without *Ampelomyces*	Samples with *Ampelomyces*
Asexual (*Oidium*)	67%	**33%**
Sexual	36%	**64%**

on those mycoparasites enabled explicit recognition of their biology and life cycle. But still, little attention has been devoted to the ecology of the *Ampelomyces* and their role in the natural environment.

In Poland, in recent years several references report on the occurrence of *Ampelomyces* on powdery mildews, including works of among others: Adamska et al. (1999) on the area of the Słowiński National Park, Czerniawska (2001a, b), Czerniawska et al. (2000) on the area of Drawski National Park and Mułenko, Wojdyło (2002) on the south of Poland or Ruszkiewicz-Michalska (2006) from the area of the Częstochowska Upland. Those investigations were usually conducted in phytocenoses and ecosystems interesting from the floristic and phytosociological point of view and characterized by a low degree of anthropopression. Often, these are protected areas with conditions similar to the natural ones, including national parks or reserves. Those works document only sporadic presence of that hyperparasites. This is most likely due to the fact that those work are inventory in character and involve the determination of species of powdery mildew and only occasional notice of their presence onto *Ampelomyces*, without assays of the infestation degree of host mycelium nor the infested morphological structures. In contrast, there are sparse works referring to the presence of *Ampelomyces* on Erysiphales in the municipal environment, which report on the high prevalence of that hyperparasites (Madej and Antoszczyczyn 1965; Sucharzewska and Dynowska 2002).

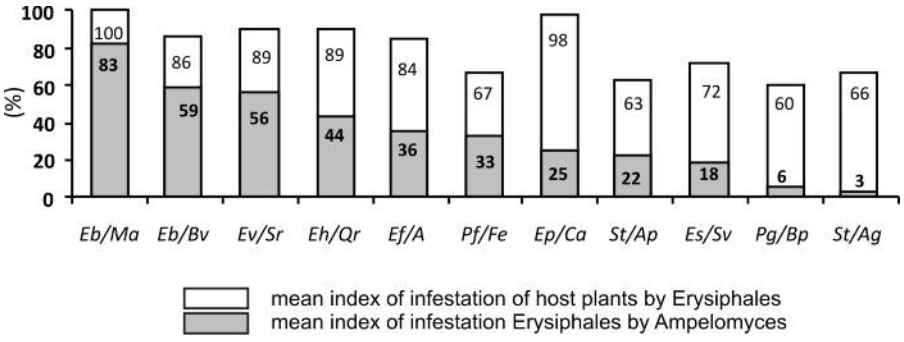
The continued own study confirmed the high prevalence of the mycoparasite on different species of powdery mildews occurring in the municipal environment. The *Ampelomyces* were noted on 9 out of 14 Erysiphales species being at the sexual stage and – to a lesser extent – on the asexual stage. In addition, analyses showed considerable differences in the prevalence of *Ampelomyces* on particular species of powdery mildews. Those hyperparasites distinctly prefer some species. The highest percentage contribution of *Ampelomyces* in the samples was noted in the case of *E. vanbruntiana var. sambuci-racemosae* (92%), *E. flexuosa* (83%) and *E. berberidis* (69%). Ruszkiewicz-Michalska (2006), in study conducted on the area of the Częstochowska Upland, did not detect the presence of that hyperparasites on the mycelium of both *E. berberidis* and *E.vanbruntiana*. Neither did she noted *Ampelomyces* on other powdery mildews: *E. hypophylla*, *E. palczewskii*, *E. syringae* and *P. guttata*, which in our study were infested by the hyperparasites. Perhaps, this is linked with the fact that observations were conducted in forest and turf communities that are not exposed to strong anthropopression. In turn, Majewski (1971) suggests, that this fungus is linked especially with the habitat strongly transformed by man, for when carrying out in-depth mycosociological analyses in the Białowieski National Park he did not detect *Ampelomyces* on powdery mildews, despite intensive searches.

Puzzling is then the lack of reports on the presence of *Ampelomyces* on Erysiphales in Polish and foreign works conducted on areas being under the influence of anthropopression (Feige and Ale-Agha 1999; Mikołajska and



Ea *– Erysiphe adunca*; ***Eal*** *– E. alphitoides*; ***Eb*** *– E. berberidis*; ***Ef*** *– E. flexuosa*; ***Eh*** *– E. hypophylla*; ***Ep*** *– E. palczewskii*; ***Es*** *– E. syringae*, ***Et*** *– E. tortilis*; ***Etr*** *– E. trifolii*; ***Ev*** *– E. vanbruntiana var. sambuci-racemosae*; ***Pf*** *– Phyllactinia fraxini*; ***Pg*** *– P. guttata*; ***St*** *– Sawadaea tulasnei*; ***Sb****-S. bicornis.*

Fig. 1. Percentage contribution of samples with the analyzed species of Erysiphales at sexual stage infected by *Ampelomyces.*



Eb/Ma *– E. berberidis on M. aquifolium*; ***Eb/Bv*** *– E. berberidis on B. vulgaris*; ***Ef/A*** *– E. flexuosa on Aesculus* spp.; ***Eh/Qr*** *– E. hypophylla on Q. robur*; ***Ep/Ca*** *– E. palczewskii on C. arborescens*; ***Es/Sv*** *– E. syringae on S. vulgaris*; ***Ev/Sr*** *– E. vanbruntiana var. sambuci-racemosae on S. racemosa*; ***Pf/Fe*** *– P. fraxini on F. excelsior*; ***Pg/Bp*** *– P. guttata on B. pendula*; ***St/Ap*** *– S. tulasnei on A. platanoides*; ***St/Ag*** *– S. tulasnei on A. ginnala.*

Fig. 2. Mean infestation degree of host plants by Erysiphales and mean infestation degree of species of Erysiphales at sexual stage by *Ampelomyces*

Dynowska 1982; Kalinowska-Kucharska and Kadłubowska 1993; Hołownia and Kostrzewska 1991).

The reported study also presents a comprehensive analysis of the extent of infestation of mycelium of the analyzed powdery mildew species by the hyperparasites. This is the first Polish study addressing that issue. In the available world literature, alike investigations – namely the evaluation of the infestation degree of mycelium of selected species of powdery mildews – were carried out by Kiss (1998) and Rankovič (1997). They reported a high percentage of *E. berberidis* mycelium infestation by *Ampelomyces.* Our study confirmed that this species of powdery mildew (along with *E. vanbruntiana* and *E. flexuosa*) is the most frequently and to the greatest extent infested by the hyperparasites analyzed. Kiss (1998) did not detect the presence of *Ampelomyces* on mycelium of three Erysiphales species: *E. alphitoides*, *E. syringae* and *S. bicornis.* In turn, in our study the hyperparasites were not observed on five species of powdery mildews, i.e.: *E. adunca*, *E. alphitoides*, *E. tortilis*, *E. trifolii* and *S. bicornis.* On that last species the hyperparasites belonging to the genus *Ampelomyces* were noted by Mułenko and Wojdyła (2002), and on *E. alphitoides* by Ruszkiewicz – Michalska (2006). In the case of *E. adunca*, *E. tortilis* and *E. trifolii*, the lack of those hyperparasites may result from a too low number of samples collected for analyses. In turn, on the last two species, the *Ampelomyces* were noted by Rankovič (1997). The presence of the hyperparasites on *E. syringae* infesting *Syringa vulgaris*, has been reported for the first time ever on that host fungi. The differences in the preference of the host fungi by hyperparasites from the genus *Ampelomyces* are likely to result from the existence of different species, confirmed by genetic research of rDNA of the ITS region (Kiss 1997; Kiss and Nakasone 1998; Kiss and Vajna 1995; Liang et al. 2007; Szentivànyi et al. 2005).

Literature reports on a considerable role of *Ampelomyces* in growth inhibition of Erysiphales (Falk et al. 1995; Füzi 2003; Madej and Antoszczyszyn 1965). In contrast, our previous investigations (Sucharzewska and Dynowska 2002) indicate that this hyperparasites did not reduce considerably the degree of infestation of a few species of powdery mildew. This has also been confirmed in a work by Kiss (1997), who was analysing the presence of *Ampelomyces* on powdery mildews infesting plants of the family *Gramineae*. He concluded on the lack of sound evidence of the effect of that hyperparasites on the impairment of sporulation of the mycelium of *Blumeria graminis* – a dangerous parasite of cereals. In the continued own study, in all the observed cases the mean degree of infestation of the host plants by powdery mildews was always higher than the mean degree of infestation of Erysiphales mycelium by the hyperparasites. This results from the fact that over the entire analytical period, the *Ampelomyces* were noted mainly at the end of the life cycle of powdery mildews, on mycelium producing fruiting bodies and – to a lesser extent – on the conidial stage. Results obtained in the reported study correspond with findings of other authors who claim that *Ampelomyces* colonizes only ageing colonies of powdery mildews, being at the teleomorphic stage in which the degree of plants infestation exceeds 60% (Füzi 2003; Gadoury and Pearson 1998; Gadoury et al. 1991).

In summary it may be concluded that the reported study proves that fungi of the genus *Ampelomyces* find very good conditions for the development on the mycelium of powdery mildews, infesting trees and bushes in the environment strongly transformed by man. They may, thus, be recognized as "urbanophilic" species. The high prevalence of the hyperparasites of that genus at, simultaneously, a high prevalence of powdery mildews suggests that under natural conditions they do not play any significant role in the reduction of the degree of infestation of host plants by Erysiphales and do not disturb drastically their life cycle.

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