POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(3): 289–299, Y. 2012

HYPERPARASITES OF *ERYSIPHALES* FUNGI IN THE URBAN ENVIRONMENT

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K e y w o r d s: *Ampelomyces,* hyperparasites, fungicolous fungi, powdery mildew, transport pollution effects, anthropopressure.

A b s t r a c t

This manuscript presents data on the occurrence of hyperparasitic fungi colonizing the mycelium of selected species of *Erysiphales*: *Erysiphe alphitoides*, *E. hypophylla, E. palczewskii, Golovinomyces sordidus, Podosphaera fusca* and *Sawadaea tulasnei* in the urban environment. In the paper the effect of hyperparasites on the development of fungal hosts at diversified level of transport pollution is emphasized. Over a three-years experiment, the presence of hyperparasites was confirmed on all analyzed *Erysiphales* species, with prevailing species from the genus *Ampelomyces.* The representatives of other genera: *Alternaria, Aureobasidium, Cladosporium*, *Stemphylium* and *Tripospermum* were also observed on mycelium of *E. alphitoides* and *E. palczewskii*. The hyperparasites occurred only on stations situated at the main roads were found not to affect the extent of plant infection by fungi of the order *Erysiphales,* but reduced the number of chasmothecia.

NADPASOŻYTY GRZYBÓW Z RZĘDU *ERYSIPHALES* W ŚRODOWISKU MIEJSKIM

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S ł o w a k l u c z o w e: *Ampelomyces*, nadpasożyty, mączniaki prawdziwe, zanieczyszczenia komunikacyjne, antropopresja.

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A b s t r a k t

Praca dotyczy występowania nadpasożytów zasiedlających plechę wybranych grzybów z rzędu *Erysiphales*: *Erysiphe alphitoides*, *E. hypophylla, E. palczewskii, Golovinomyces sordidus, Podosphaera fusca i Sawadaea tulasnei* w warunkach miejskich. Oceniono wpływ nadpasożytów na rozwój grzybów-gospodarzy, ze zwróceniem szczególnej uwagi na zanieczyszczenia komunikacyjne. Podczas trzyletnich badań na wszystkich analizowanych gatunkach *Erysiphales* stwierdzono obecność nadpasożytów z przewagą gatunków z rodzaju *Ampelomyces*. Najwyższe porażenie mączniaków prawdziwych odnotowano na stanowiskach znajdujących się przy głównych szlakach komunikacyjnych. Na grzybni *E. alphitoides* i *E. palczewskii* odnotowano również występowanie grzybów z rodzajów *Alternaria, Aureobasidium, Cladosporium*, *Stemphylium* i *Tripospermum*, które występowały tylko w punktach zlokalizowanych do 50 m i 100 m.

Nadpasożyty nie wpływały na średni stopień porażenia roślin mączniakiem prawdziwym, natomiast redukowały liczbę owocników.

Introduction

Hyperparasitism is a phenomenon that plays a significant though underestimated role in the dynamics of fungi population in the natural environment (JEFFRIES 1995). However, studies on hyperparasites are mainly focused on the search for microorganisms effective in the biological fight against plant pathogens.

Results of many research addressing economically important phytopathogens – powdery mildews (*Erysiphales*), report on the occurrence on the mycelium of numerous antagonistic fungi, including: *Tilletiopsis* sp. Derx (URQUHART et al. 1994), *Sporothrix flocculosa* Traquair, Shaw & Jarvis (ELAD 1995), *Lecanicillium lecanii* (Zimm.) Zare & W. Gams [= *Verticillium lecanii* (Zimm.) Viégas] (ASKARY et al. 1998), *Phoma glomerata* (Corda) Wollenw. & Hochapfel (SULLIVAN, WHITE 2000), *Acremonium alternatum* Link (ROMERO et al. 2002), *Cladosporium uredinicola* Speg. (HEUCHERT et al. 2005) or fungi of the genus *Ampelomyces* Ces. ex. Schltdl. The latter, acknowledged to be endoparasites, have been recognized as the major antagonists of powdery mildews and thus have been the most frequent subject in this field of interest. Fungi of the genus *Ampelomyces* are widespread worldwide and were recorded on 66 host species of fungi, belonging to 9 genera (KISS et al. 2004). The ability of these antagonistic fungi to colonize the mycelium of many *Erysiphales* species, to restrict their sporulation as well as to inhibit the formation and development of chasmothecia, has been successfully used in practice. A fine example in this case is a biofungicide AQ10, applied in plant protection against powdery mildews (KISS et al. 2004).

Polish literature provides quite comprehensive information on the occurrence of fungi of the genus *Ampelomyces* on the powdery mildews. So far, the hyperparasites were recorded on 44 species of *Erysiphales* from 6 genera: *Erysiphe, Microsphaera, Podosphaera, Sphaerotheca, Uncinula* oraz *Oidium*

(MUŁENKO et al. 2008, erysiphalean nomenclature following Braun 1995). Most papers documented mainly the presence of *Ampelomyces* pycnidia on mycelium of different host species and occasionally reported either symptoms of infestation or the infected morphological structures. However, far too little attention has been paid to the ecology and ecophysiology of hyperparasites developing under natural conditions (ADAMSKA, CZERNIAWSKA 2010, CZERNIAWSKA 2005, CZERNIAWSKA et. al. 2000, CZERNIAWSKA, ADAMSKA 2007, MUŁENKO, WOJDYŁO 2002, RUSZKIEWICZ-MICHALSKA 2006). Especially their effect on the development of host fungi in the natural environment is poorly understood (ADAMSKA, CZERNIAWSKA 2010, KISS 1997, 1998, MAJEWSKI 1971, RANKOVIČ 1997). The available information about an occurrence of the hyperparasites in urbicoenoses is insufficient (CZERNIAWSKA et. al 2011, MADEJ, ANTOSZCZYSZYN 1965, RUSZKIEWICZ-MICHALSKA 2010, RUSZKIEWICZ-MICHALSKA, MICHALSKI 2005, SCHMIDT, SCHOLLER 2002, SUCHARZEWSKA, DYNOWSKA 2005, SUCHARZEWSKA et al. 2011), especially the one covering the impact on the development of chasmothecia of *Erysiphales* members. Research works addressing this issue are usually focused on particular, economically important pathogens, for instance powdery mildew of grape – *Erysiphe necator* Schwein [=*Uncinula necator* (Schwein.) Burrill] (ANGELI et al. 2009, FALK et al. 1995, GADOURY, PEARSON 1988, FÜZI 2003).

This paper presents a part of results of study on life strategies of selected *Erysiphales* species carried out in the years 2000-2002 in the urban habitats diversified according to the level of transport pollution (SUCHARZEWSKA 2009, 2010, SUCHARZEWSKA, DYNOWSKA 2005). The aim of this study was to analyze the occurrence of hyperparasites and their effect on the development of selected fungi hosts in the above mentioned conditions.

Material and Methods

Six species of powdery mildews were selected for the study: *Erysiphe alphitoides* (Griffon et Maubl.) U. Braun et S. Takam. and *E. hypophylla* (Nevod.) U. Braun et Cunningt. on *Quercus robur* L., *E. palczewskii* (Jacz.) U. Braun et S. Takam. on *Caragana arborescens* Lam., *Golovinomyces sordidus* (L. Junell) V.P. Heluta on *Plantago major* L., *Podosphaera fusca* (Fr.) U. Braun et Shishkoff on *Taraxacum officinale* F. H. Wigg. and *Sawadaea tulasnei* (Fuckel) Homma on *Acer platanoides* L.

The experiment was carried out in three vegetative seasons (2000–2002) in the city of Olsztyn and its vicinity north-eastern Poland. Research sites (175 localities), were situated at a range of distances from the main transport routes: up to 50 m, up to 100 m, up to 300 m, and >300 m used as a control station. These distances were selected based on a study by LORENC-PLUCIŃSKA and BYCZYŃSKA (1997), which shows that the greatest value of exhaust gas concentration is recorded at a distance of 30–50 m away from the road and maintains at a level of up to 30%. The exhaust gas level drops to 10% at 200 m away from transport routes.

The material in the form of infested plants was collected every three weeks throughout the study period. In the case of *A. platanoides*, *C. arborescens* and *Q. robur* – single sample was defined as 25 leaves collected randomly from one host plant. In the case of *P. major* and *T. officinale* – single sample consisted of 10 leaves collected randomly from a 1 m^2 area covered with the host plant.

At the laboratory, the collected material was subjected to macro- and microscopic analyses:

1. To calculate the disease index (R) of hosts infected by fungi McKinney's formula (5° scale) – (MCKINNEY 1923) was used:

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

R_E – denotes disease index of plants infested by *Erysiphales* fung i while (R_A and R_H) concern the disease index of powdery mildews' mycelium infested by *Ampelomyces* species (exclusively) and by other hyperparasites (all species treated together), respectively.

Σ (*ab*) · 100% – the sum of products obtained by multiplying the number of analyzed: organs of plants (*a*) by the degree of infection with parasite (*b*); in the case of hyperparasites the degree of infection is referred to the number of plants infected by powdery mildew host.

N – the total number of plant organs examined / the total number of organs of plants infected by powdery mildew,

4 – the highest degree of infection.

Degree of infestation was estimated according to a five-grade scale: 0 – no infection; 1 – up to 10% of leaf area; 2 – 11–25%; 3 – 26–50%; 4 – 51–100%.

2. Analyses were also conducted for the effect of hyperparasites on the number and development of chasmothecia:

a) in twenty samples selected at random from each *Erysiphales* species that proved to be hyperparasitized; the number of chasmothecia was set on randomly selected 1 cm^2 area of each leaf covered by powdery mildew mycelium in two variants: 1) with hyperparasites and 2) without them.

b) 10 mature chasmothecia (with dark peridium) selected at random from each sample were analyzed for:

– developmental stage of ascocarp appendages estimated according to a three-grade scale: **0** – lack of appendages, **I** -not fully developed and **II** – fully developed,

– the presence of asci and ascospores.

Olympus SZX9 stereomicroscope and Olympus BX41 light microscope were used to observe a mycelium, chasmothecia and hyperparasites' structures (pycnidia, conidiophores and conidia).

The final values provided in the Results section were computed based on an arithmetic mean determined for each fungi species, on a specified host plant. In the case of *E. alphitoides* and *E. hypophylla,* the mean degree of infection was calculated as a total value for these species due to their co-occurrence (SUCHARZEWSKA 2009).

Fungi were determined using keys by BRAUN (1995), SAŁATA (1985), ELLIS and ELLIS (1985) and ELLIS (1971). The erysiphalean nomenclature was accepted after BRAUN and TAKAMATSU (2000) as well as BRAUN et al. (2003); other fungal names follow Index Fungorum (www.indexfungorum.org). The nomenclature of host plants was adopted after MIREK et al. (2002).

Results

In total, 867 samples of powdery mildews were collected. In 433 samples (50%) fungi of the genera (sensu lato treatment): *Alternaria* Nees*, Ampelomyces* Ces. ex Schltdl.*, Aureobasidium* Viala, et G. Boyer*, Cladosporium* Link*, Stemphylium* Wallr. and *Tripospermum* Speg. were recorded.

Samples with fungi of the genus *Ampelomyces* had the highest percentage (92%) among the samples containing hyperparasites. They colonized powdery mildews' mycelium to a different extent. *Ampelomyces* fungi were dominant in samples of *Golovinomyces sordidus* – 48% and *Podosphaera fusca* – 45%. In the case of the other species, the percentage of *Ampelomyces* hyperparasites in samples ranged from: 25% in *E. palczewskii*, 19% in *S. tulasnei* and 15% in *E. hypophylla* to 2% in *E. alphitoides*. The presence of the *Ampelomyces* fungi was observed not to affect the mean index of plants infection by *Erysiphales* species (R_E). In all cases, R_E values were higher than the disease indexes of powdery mildews mycelium infested by the analyzed hyperparasites (R_A and R_H) – Table 1. The highest disease index of mycelium (R_A) was noted at stations located up to 50 m and 100 m away from polluters. In contrast, at the control station and others located up to 300 m away, no hyperparasite or a low values of diseases index were observed.

Table 1

Mean index of infestation of host plants by powdery mildews and mean index of infestation of *Erysiphales* species by hyperparasites at various distances from roads

Species of *Erysiphales*		Distances from roads			
		up to 50 m	up to 100 m	up to 300 m	>300 m
E. alphitoides	R_E	44	52	36	34
	R_A	0	0	0	0
	R_H	34	22	0	0
E. hypophylla	R_E	44	52	36	34
	R_A	2	1	1	1
	R_H	0	0	0	0
E. palczewskii	R_E	55	68	47	14
	R_A	12	20	0	0
	R_H	25	0	0	0
G. sordidus	R_E	47	46	31	27
	R_A	42	31	18	17
	R_H	0	0	0	0
P. fusca	R_E	45	43	17	7
	R_A	39	38	7	3
	R_H	0	0	0	0
S. tulasnei	R_E	23	20	13	1
	R_A	16	19	1	0
	R_H	0	0	0	0

Explanations:
R_E – Mean index of host plants infestation (%) with powdery mildews; R_A – Mean index of powdery mildew infestation (%) with Ampelomyces *species;* R_H – Mean index of powdery mildew infestation (%) with hyperparasites from other genera.

Dark colored mycelium and conidiomata of the fungi from genera: *Alternaria, Aureobasidium, Cladosporium, Stemphylium* and *Tripospermum* were developed on the mycelium and chasmotecia of their host fungi. Hyperparasite' mycelium was also visible inside of appendages of *E. palczewskii* chasmothecia. In total, those hyperparasites constituted about 8% of all samples containing hyperparasites, predominating in samples of *Erysiphe palczewskii* (23%) and *E. alphitoides* (18%). In the case of the other *Erysiphales* species, the fungi occurred sporadically. Very often species of *Alternaria, Aureobasidium, Cladosporium, Stemphylium* and *Tripospermum* co-occurred on the host and were noted at stations located up to 50 and 100 m away from transport routes (Table 1).

The hyperparasites were found to affect the number of mature chasmothecia of powdery mildews. *Ampelomyces* species were observed to reduce the number of chasmothecia 7 times in case of *E. hypophylla* and 6 times in *E. palczewskii*, 5 times in *G. sordidus* and *P. fusca* as well as 3 times in *S. tulasnei* (Figure 1). Over 4-fold reduction in the mean number of chasmothecia was noted in *E. palczewskii,* and above 5-fold in *E. alphitoides* which mycelium was colonized by the other hyperparasites (Figure 1).

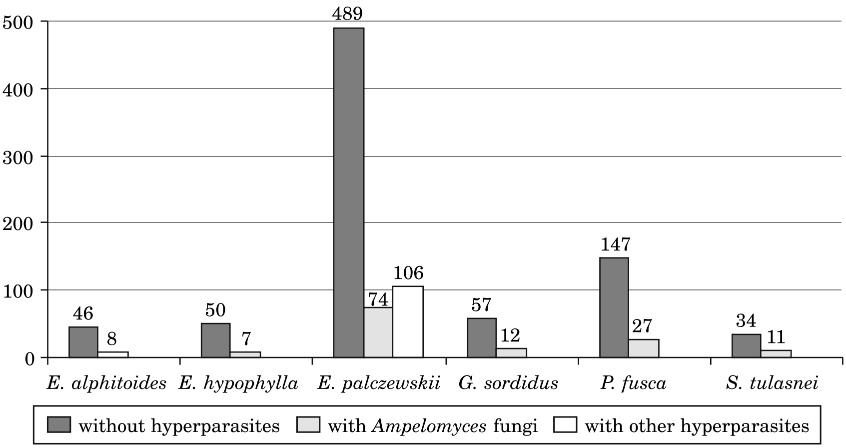

Fig. 1. The mean number of powdery mildews' ascocarps on 1 cm^2 of leaf surface without and with hyperparasites

The comparative analysis of mature chasmothecia morphology produced by infected and non-infected mycelium demonstrated no developmental disorders in the case of *Ampelomyces* fungi: mature chasmothecia had well developed appendages, asci and spores. In contrast, a negative effect on their development was observed in infection of *E. palczewskii* mycelium caused by other hyperparasitic fungi. A considerably lower percentage (32%) of appendages at II developmental stage was noted as compared to chasmothecia of the non-infected mycelium (91%). In addition, chasmothecia without asci and ascospores were more numerously present (11% of all samples) at the mycelium infected by parasites than at non-infected (1%).

Discussion

There is no group of living organisms that could avoid parasites (COMBES 1999). In the reported study, the presence of hyperparasites was recorded on mycelium of all of analyzed species of phytopathogenic fungi. Hyperparasites were most frequently the representatives of the genus *Ampelomyces*, widely regarded as the major antagonist of powdery mildews. Only the sporadic occurrence of *Ampelomyces* fungi on powdery mildew mycelium was reported in Polish literature covering investigations conducted in national parks and arboreta (ADAMSKA et al. 1999; CZERNIAWSKA et. al. 2000, MUŁENKO, WOJDYŁO 2002). MAJEWSKI (1971) suggested that *Ampelomyces quisqualis* Ces. is linked with the anthropogenic environment. MAJEWSKI (1971) based his opinion on the data and experience gathered during in-depth mycosociological research he conducted in the Białowieża National Park. Despite intensive search those fungi was not found on powdery mildews in natural phytocoenosis. However *Ampelomyces* hyperparasites were often noted on numerous powdery mildew species in the cities of Szczecin and Łódź (MADEJ, ANTOSZCZYSZYN 1965, RUSZKIEWICZ-MICHALSKA, MICHALSKI 2005, RUSZKIEWICZ-MICHALSKA 2010). Our study confirms frequent occurrence of the hyperparasites in the urban environment.

The highest degree of infection by *Ampelomyces* hyperparasites proved at stations located closed to (50 m and 100 m) the main city transport routes resulted from a high disease index of the plants. In addition, *Ampelomyces* species occurred sporadically at stations with a low value of the index of plants infection. Our study demonstrates also that the mean index of host plants infection by powdery mildews was always higher than the mean index of *Erysiphales* mycelium infection by *Ampelomyces*. It indicates that the spread of their hosts in urban environment is not affected by the hyperparasites too drastically. The same conclusion has been drawn from the results of another of our studies dealing with hyperparasites of numerous *Erysiphales* species in urban environment (SUCHARZEWSKA et al. 2011).

Currently observed differences in the preference of *Ampelomyces* fungi to some species of host fungi may, presumably, result from the presumed genetical heterogeneity of the genus *Ampelomyces* considered by some authors to be rather a complex of taxa than a single species (KISS, VAJNA 1995, KISS, NAKASONE 1998). The differences in the mode of occurrence of those hyperparasites and disparate values of disease index were also observed by KISS (1998).

Results of the reported study addressing the effect of *Ampelomyces* fungi on the number of produced chasmothecia correspond with findings of GADOURY et al. (1991), FÜZI (2003) and ANGELI et al. (2009). The reduction in the number of chasmothecia recorded for all representatives of *Erysiphales* results from colonizing young chasmothecia and transforming them into own reproductive structures of the hyperparasite.

Apart from *Ampelomyces* fungi around forty of other natural antagonists of *Erysiphales* representatives are known (KISS 2003). Under laboratory conditions, some of these fungi (e.g. *Acremonium alternatum* and especially *Lecanicillium lecanii*) were observed to inhibit the growth of *Podosphaera fusca* [= *Sphaerotheca fusca* (Fr.) Blumer] more effectively than the *Ampelomyces* fungi (ROMERO et al. 2002). Various other fungi, e.g. species of *Alternaria, Botrytis, Candida, Cephalosporium, Cladosporium* and *Trichothecium* are commonly found within the mycelial colonies of the powdery mildews (BRAUN 1995). KISS (2003) reported colonization of chasmotecia of *Phyllactinia* spp. and reduction of their number by fungi from the genus

Cladosporium. The latter may occur on the leaf area together with other fungi such as *Aureobasidium pullulans*, forming a complex called '*Fumago vagans*' (ELLIS and ELLIS 1985). In Poland representatives of the genus *Alternaria* and *Cladosporium* on mycelium of some *Erysiphales* species were observed by ADAMSKA and CZERNIAWSKA (2010) while PIĄTEK (2003) reported the occurrence of *Alternaria* sp. on *Phyllactinia fraxini* (D.C.) Fuss. On the same host species *Cladosporium uredinicola* and *Phoma glomerata* have been recently reported (RUSZKIEWICZ-MICHALSKA 2010). Complex '*Fumago vagans*' was observed on the mycelium of *S. tulasnei*, in our study while no *Phoma* species was confirmed in the material studied.

The presence of anamorphic fungi of genera *Alternaria, Aureobasidium, Cladosporium*, *Stemphylium* and *Tripospermum* on the mycelium of *Erysiphe alphitoides* and *E. palczewskii* may be due to a high level of traffic pollution. Polish research on the occurrence of fungi in a variety of stressful conditions provide interesting information about a burst of activity of some phytopathogens. The intense development of fungi from the genus *Alternaria*, *Aureobasidium* and others on plants of industrialy polluted sites was reported by DOMAŃSKI et al. (1977). Those fungi are referred to as saprotrophs that may infect of plants debilitated due to the detrimental effects of the environment. In our study those fungi disrupted development of *E. palczewskii* chasmothecia, that confirms their possibly negative impact. Presence of hyphae of antagonists inside of appendages of *E. palczewskii* chasmothecia points to internal interference – that according to JEFFRIES (1995) indicates invasive necrotrophy. A similar phenomenon was observed by HOCH and PROVIDENTI (1979) in *Sphaerotheca fuliginea* attacked by *Tilletiopsis* sp. This hyperparasite surrounded the hyphae of host causing their necrosis and penetrating them.

In summary, the reported study confirmes the phenomenon of hyperparasitism on fungi of the order *Erysiphales* as widespread in the urban environment. The predomination of antagonistic fungi is found to be linked directly with the high degree of plants infestation by powdery mildews. This seems to enhance the development of antagonistic fungi which, in turn, significantly affect the powdery mildews population dynamics by reducing the number of vital chasmothecia.

Translated by JOANNA MOLGA

Accepted for print 16.07.2012

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