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The development of *Erysiphe alphitoides* and *E. hypophylla* in the urban environment

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Differentiated responses of *Erysiphe alphitoides* and *E. hypophylla* in urban conditions are described. The influence of transport pollution on the morphology of the mycelium, chasmotecium development and individual stages of the developmental cycle is discussed.

Key words: *Erysiphales,* developmental cycle, transport pollution, Olsztyn

INTRODUCTION

Erysiphe alphitoides Griff et Maubl. and *E. hypophylla* Nevod. are common obligate parasites of the order *Erysiphales*. They infect representatives of the genera *Quercus* and *Fagus* as well as sporadically *Aesculus* and *Castanea. Paeonia lutea* has been identified as a new host of *Erysiphe hypophylla* (Takamatsu et al. 2006). The two phytopathogens are widespread in entire Europe, Asia, America, Australia and New Zealand. The occurrence of *Erysiphe alphitoides* has been documented in Poland since 1909, initially in the conidial stage only (*Oidium quercinum* Thüm). The sexual stage of *E. alphitoides* was first observed in France in 1911 and recorded in Poland in 1922 (Braun 1995; Sałata 1985). It has since been recorded in the teleomorphic stage as *Microsphaera alphitoides* very often (Adamska et al. 1999; Czerniawska 2001; Dynowska et al. 1999; Kalinowska-Kucharska, Kadłubowska 1993; Kućmierz 1967, 1971, 1973; Majewski 1970, 1971; Mułenko 1981, 1988; Mułenko, Wojdyło 2002; Ruszkiewicz-Michalska 2006; Sałata et al. 1993). *Erysiphe alphitoides* is a dangerous oak pathogen. It attacks trees of various age classes causing a reduction in annual growth (Minkievič et al. 1993).

Erysiphe hypophylla was first observed in Poland in 1952 (Sałata 1985). It has been recorded sporadically in different parts of Poland (Dynowska et al. 1999; Majewski 1971; Mułenko 1988; Sałata, Majewski 1976).

In contrast to *Erysiphe alphitoides*, the systematic position of *E. hypophylla* was not clear until recently (Braun, Takamatsu 2000). The species was considered by many mycologists to be a synonym of *Erysiphe alphitoides* (*Microsphaera alphitoides*) based on biometric studies. Braun et al. (2003) finally distinguished two separate species using molecular studies: *Erysiphe alphitoides* and *Erysiphe hypophylla*.

The two fungi, mostly *Erysiphe alphitoides* and less frequently *E. hypophylla*, has been reported in studies investigating their presence in floristically or phytosociologically interesting communities and ecosystems characterised by a low level of anthropopressure. As they were often inventories or phytopathological examinations, these studies usually provided systematic and geographical analyses of species richness or an assessment of taxonomic differentiation in relation to plant associations in a given area (Adamska et al. 1999; Czerniawska 2001; Majewski 1971; Mułenko 1996, 1998; Kućmierz 1971, 1973). However, the ecology *Erysiphe alphitoides* and *E. hypophylla* and their life strategies in environments exposed to strong anthropopressure have been discussed in few studies.

The aim of this study was to analyse the occurrence of *Erysiphe alphitoides* and *E. hypophylla* and to examine basic life reactions of the parasites in urban conditions.

MATERIAL AND METHODS

Examinations (2000-2002) were conducted in the city of Olsztyn and its vicinity at 63 sites (17 sites with young plants and 46 sites with mature *Quercus robur* L. plants) situated at a range of distances from main transport routes: up to 50 m, up to 100 m, up to 300 m and >300 m. Distances were selected using studies by Lorenc-Plucińska and Byczyńska (1997) which show that the greatest value of exhaust gas concentration is recorded at a distance of 30-50 m away from the road and is at a constant level of 30%. The exhaust gas level drops to 10% at 200 m away from transport routes. Sites located >300 m were used as controls.

Material was obtained from the middle of the vegetative period when first chasmothecia began to occur on the powdery mycelium. One sample was defined as a total of 25 leaves collected randomly from each host plant.

The infection rate of host plants, mycelium development and morphology as well as chasmothecium development were examined in the macro- and microscopic analysis of the material.

1. The disease rating was calculated for each sample on a 5°-scale using McKinney's formula. It shows the degree of infection of host plants and is a criterion of pathogen sensitivity (Dynowska 1993, 1994) to changes in the optimum environment conditions:

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

R – disease coefficient in percent (index); Σ (a x b) x 100% – the sum of the products obtained by multiplying the number of plant organs (a) by the degree of infection (b); N – total number of plants (alternatively leaves, fruits) examined in the study; 4 – the highest degree of infection in a five-grade scale (0 – no infection; 1 – up to 10%; 2-11 – 25%; 3-26 – 50%; 4-51 – 100%).

The final value R used in the results analysis was calculated based on the arithmetic mean for each fungal species on a specific host plant = mean degree of infection.

2. The developmental stage of the parasite was identified: asexual stage, sexual stage.

3. The number of chasmothecia, both mature and immature, per 1 cm^2 of the surface of each infected leaf was established. Ten randomly collected morphologically mature chasmothecia were selected and analysed under a microscope. The following were assessed:

a) developmental degree of appendages in a three-grade scale: 0 – chasmothecia without appendages, I – chasmothecia with appendages not fully developed, II – chasmothecia with fully developed appendages;

b) maturity of chasmothecia in a three-grade scale: 0 – chasmothecia without asci and spores; I – chasmothecia with asci without formed ascospores; II – chasmothecia with asci containing spores;

c) morphological variability of chasmothecia, the size and diameter of chasmothecia, appendages.

The number of chasmothecia discussed in the results was calculated based on the arithmetic mean for each fugal species.

Fungi were determined using keys by Braun (1987, 1995) and Sałata (1985). Species were identified using morphological traits of chasmothecia (size and diameter, number of appendages, appendages branching).

The nomenclature was accepted after Braun and Takamatsu (2000) and Braun et al. (2003). Host plants were determined using studies by Szafer et al. (1988) and Rutkowski (1998).

Statistical calculations were conducted using the data analysis software system STATISTICA (StatSoft, Inc.) version 6 (2003) by variance analysis. Significant differences among the means were declared at a significance level of $p=0.05$. The means were then classified in homogenous groups for selected factors using Duncan's test. Letter symbols (a, b, c…) were used and values to which the same letters were assigned do not differ significantly at $p=0.05$.

RESULTS

Erysiphe alphitoides and *E. hypophylla* were observed on both mature and young *Quercus robur* plants in all the years of the study period: at 40 sites in 2000 (63%), at 47 sites in 2001 (75%) and at 48 sites in 2002 (77%) (Fig.1).

The analysis of the degree of infection of the host plant in relation to the distance from transport routes shows noticeable, statistically significant differences. The highest mean disease index was recorded at sites located up to 100 m and 50 m (52% and

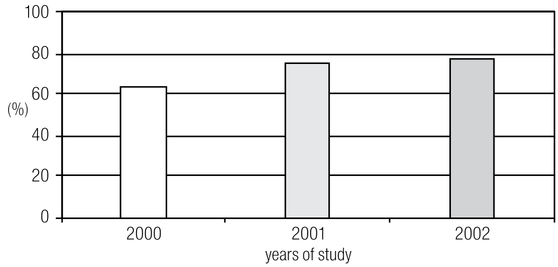

Fig. 1. Percentage participation of *E. alphitoides* and *E. hypophylla* in samples on *Q. robur*.

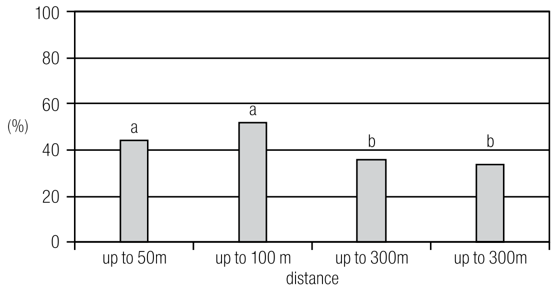

Fig. 2. Mean degree of infection of *Q. robur* by *E. alphitoides* and *E. hypophylla* in relation to the distance from transport routes in the study period (letters a and b indicate statistically significantly different values at p=0.05).

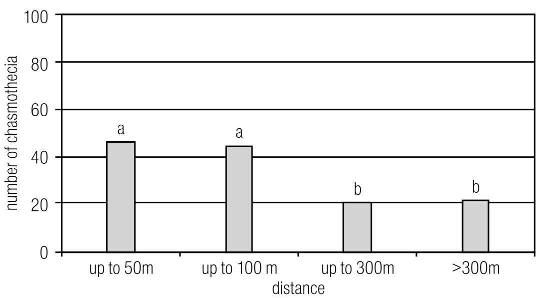

Fig. 3. Mean number of *E. alphitoides* chasmothecia per $1cm^2$ of leaf surface throughout the study period.

44%, respectively). The lowest mean infection was observed at sites located up to 300 m and >300 m, which is confirmed by the statistical analysis (Fig. 2).

The two parasites either occurred separately or co-occurred on the same plant and even on the same leaf. The upper leaf surface was usually colonised by *E. alphitoides* and the lower leaf surface by *E. hypophylla*. Chasmothecia of *E. alphitoides* were recorded beside chasmothecia of *E. hypophylla* on the lower leaf surface in a few cases.

A relatively high participation of the species' co-occurrence on the same leaves was recorded in each study year. Changes in the prevalence of the two fungi were also observed. *E. alphitoides* increased its occurrence at the distances examined in the study year by year. The highest participation of the species was recorded at sites located up to 50 m. *E. hypophylla* occurred more frequently at sites located up to 300m and >300m (Fig. 4).

Two developmental stages of *E. alphitoides* and *E. hypophylla* were observed throughout the study period: the asexual stage when conidial spores occurred and the sexual stage when chasmothecia were produced. Samples containing the perfect stage of the two fungi constituted the highest percentage regardless of the distance (>70%).

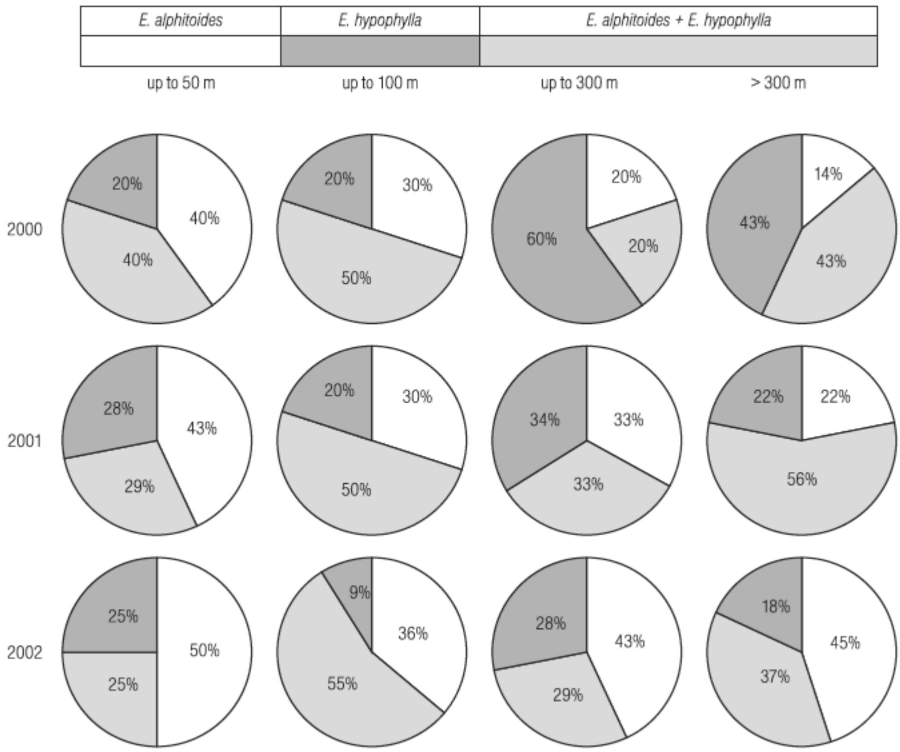

Fig. 4. Percentage participation of *E. alphitoides* and *E. hypophylla* samples at various distances throughout the study period.

The analysis of the relationship between the mean number of chasmothecia per 1 cm^2 of leaf surface and the site distance shows statistically significant results only in the case of *E. alphitoides*. The highest means were obtained for 50 m-47 ch./cm^2 and the lowest means for 300 m-20 ch./cm^2 (Fig. 3).

Chasmothecia were always in different developmental stages, from young (white, yellow and orange) to mature (brown and dark brown), in both species in all the zones examined in the study. Significant differences in the percentage participation of young and mature chasmothecia depending on the distance were not observed either in *E. alphitoides* or in *E. hypophylla*.

Different developmental stages of appendages in morphologically mature chasmothecia (dark brown chasmothecia were defined as mature) were recorded in all the zones. In *E. alphitoides*, chasmothecia without appendages (0 stage) and appendages in the first developmental stage, not fully developed, constituted the greatest percentage (Fig. 5).

However, the greatest participation of appendages in the first (I) and second (II) developmental stages was observed in *E. hypophylla*. Chasmothecia without appendages constituted a very small percentage in all the zones throughout the study period (Fig. 6).

No relationship between the level of appendage development in chasmothecia and the distance from transport routes was shown statistically. The correlation analysis

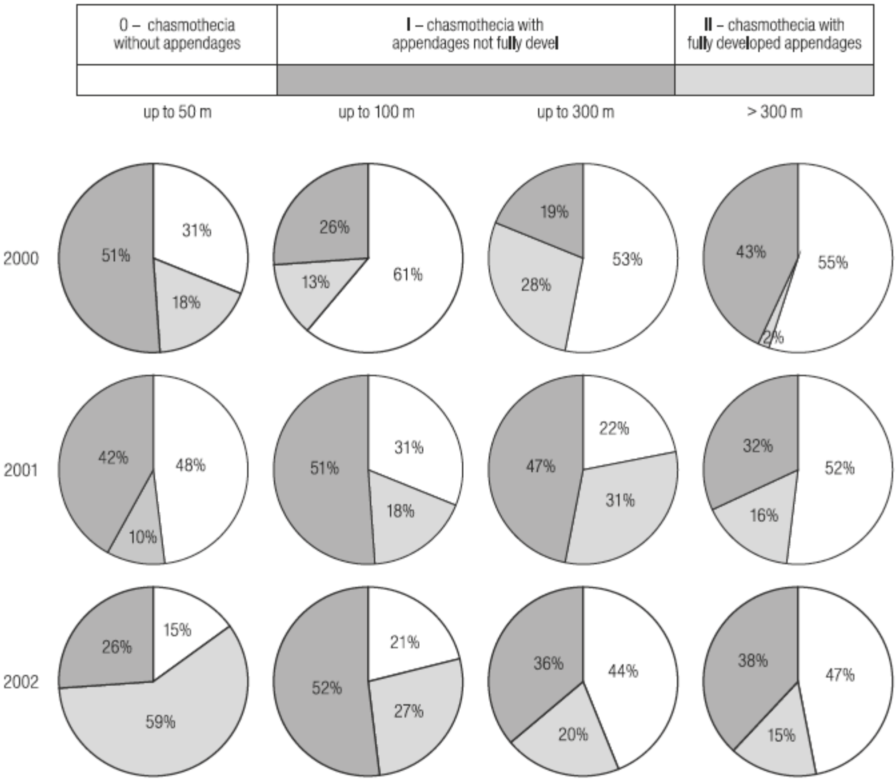

Fig. 5. Percentage participation of chasmothecia having appendages in various developmental stages in *E. alphitoides*.

showed that the participation of chasmothecia of *E. alphitoides* and *E. hypophylla* having developed appendages increased as the mean number of chasmothecia increased.

Chasmothecia having untypical appendages characterised by abnormal branching (branching hypha-like and branched dichotomously at half the length) were observed in a few cases in *E. alphitoides* at 50 m and 100 m. They constituted only 1%. The statistical analysis showed a relationship between the number of chasmothecia per $1cm^2$ and the presence of untypical appendages. The number of chasmothecia having untypical appendages increased as the number of chasmothecia increased.

The analysis of asci and ascospores did not show statistically significant differences in relation to the distance from transport routes. However, distinct interspecific differences were observed. Chasmothecia containing asci and spores as well as chamsothecia containing asci without ascospores had the greatest participation in the majority of cases in *E. alphitoides*. The participation of chasmothecia without asci and spores was also high (Fig.7). Chasmothecia without asci and spores (stage 0) constituted a very low percentage (<4%) in *E. hypophylla* (Fig. 8).

The correlation analysis showed that the participation of chasmothecia with asci containing ascospores increased together with the mean number of chasmothecia, an increase in the number of mature chasmothecia and an increase in the number of chasmothecia having appendages in stages I and II of development in *E. alphitoides* and *E. hypophylla*.

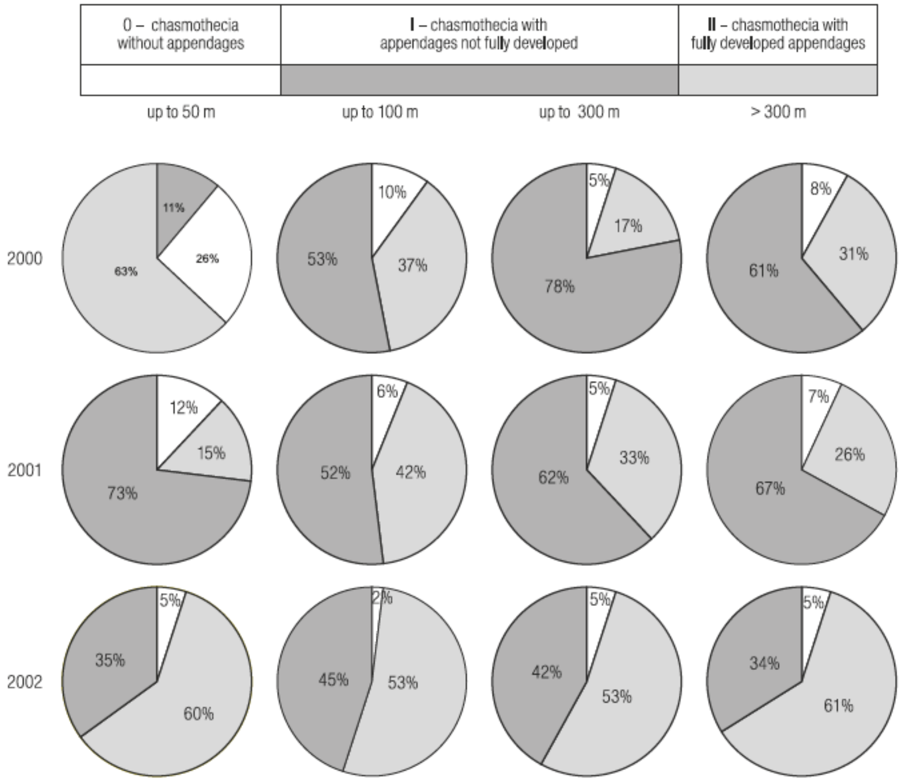

Fig. 6. Percentage participation of chasmothecia having appendages in various developmental stages in *E. hypophylla*.

Stages 0 and I of appendage development were always observed in chasmothecia that had not produced asci and spores in both species. However, untypical chasmothecia that had normally developed appendages in stage II and did not contain asci and spores were observed among perithecia of *E. alphitoides* at sties located at 50 m away from transport routes. They were considerably smaller than typical chasmothecia: their size ranged from 41μm to 64 μm, the number of appendages was between 3 and 12, and appendage length ranged from 92 μm to 153 μm. The greatest mean number of chasmothecia was also recorded at these sties.

DISCUSSION

Strong disease symptoms caused by pathogenic fungi are not observed in natural communities where the biological balance is preserved (Mułenko 1998). Progressing human-induced degradation of the environment leads to an imbalance in adaptation mechanisms of all its components and, as epiphytoses show, seriously upsets the operation of biological systems. The intensification of some fungal diseases, including

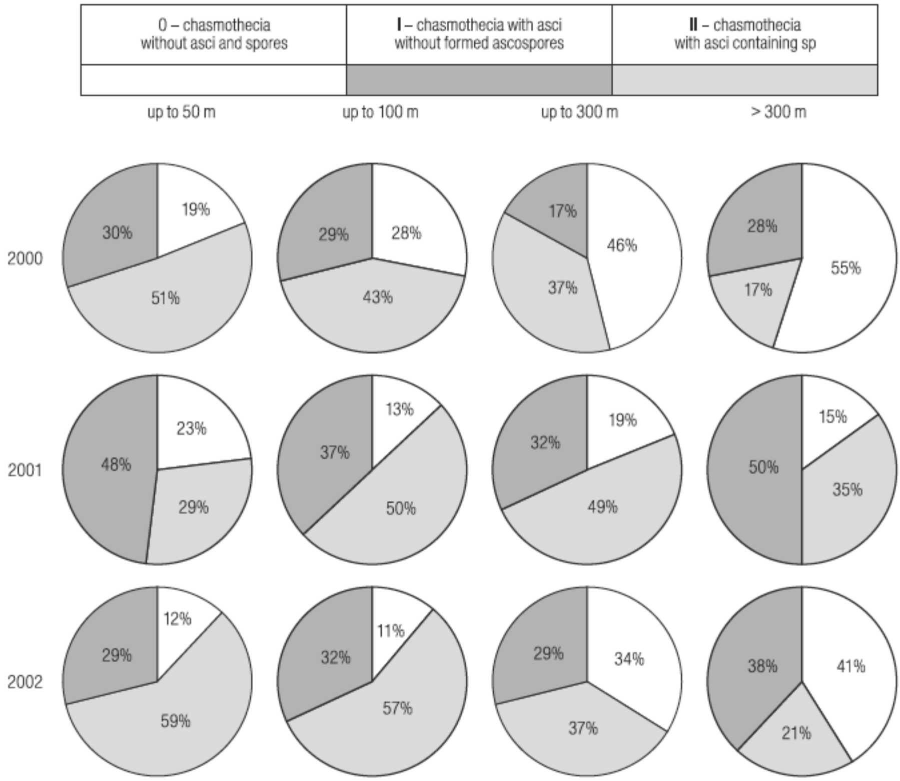

Fig. 7. Percentage participation of chasmothecia having different degrees of maturity in *E. alphitoides.*

those caused by powdery mildews, in environments affected by human intervention in recent years has been reported in studies by Jarvis et al. (2002) and Knops et al. (1999). Several fungal species fully realise their life functions in the environment of the city, often described as an urbicenosis (Sudnik-Wójcikowska 1998). Urbanophiles are defined Sudnik-Wójcikowska as organisms that find urban conditions favourable for development, in contrast to urbanophobes. The present study shows that *Erysiphe alphitoides* and *E. hypophylla* are urbanophiles. Both species occurred in each year of the study period and a rise in the activity of *Erysiphe alphitoides* was observed at the most polluted sites where the degree of infection of the host plant could reach up to 100%. Present results on the occurrence of *Erysiphe alphitoides* on oaks do not correspond with studies by some authors who considered the species to be sensitive to various types of pollution, both transport and industrial pollution. The absence of the parasite in the vicinity of motorways was observed by Flückiger and Oertli (1978). Dynowska (1996, 1996a) recorded a considerably lower degree of infection of oaks in the vicinity of transport routes in different cities and towns in north-eastern Poland in comparison with regions outside urban areas. A low degree of *Q. robur* infection by *E. alphitoides* or the disappearance of the species in species polluted by the industry are reported by Benben & Sierota (1976), Grzywacz & Ważny (1973) and Kowalski & Wrzalik (1996). The latter believe that pollution

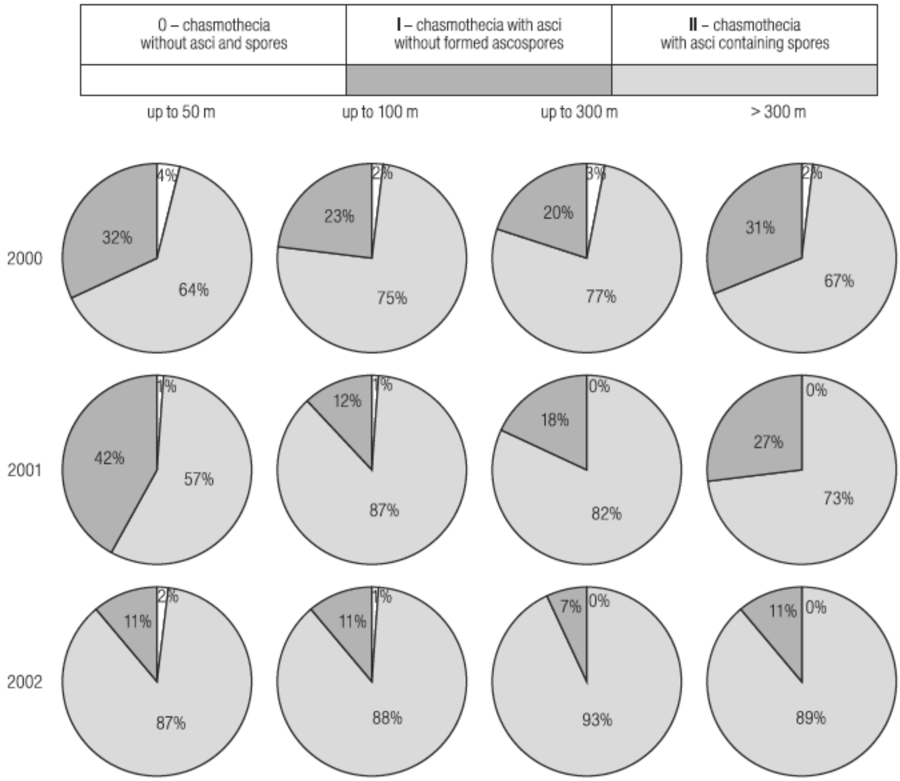

Fig. 8. Percentage participation of chasmothecia having different degrees of maturity in *E. hypophylla*.

indirectly influences the disappearance of the powdery mildew on oaks while it has a direct impact on the condition of the host plant. Dynowska (1996a) suggests that adaptation processes of the entire parasite-host system should be analysed. Reduced immunity of host plants cannot be excluded as the success of the initiation of the parasitic contact and the full development of the parasite depend on the host's health condition.

A high level of oak infection recorded in the present study is likely to result from the poor health condition of the plants in urban conditions. A noticeable downward trend in the level of sulphur dioxide in air has been observed in Olsztyn in the last few years. However, a steady, high level of nitrogen dioxide concentration (NO_2) which, as tests conducted by the Voivodeship Sanitary and Epidemiological Station in Olsztyn show, reaches highest values at very busy junctions and is considerably lower on the outskirts and outside the city, is alarming. Automotive exhaust gases distrub basic life processes in plants (Lorenc-Plucińska, Byczyńska 1997) while nitrogen compounds diluted in plant tissues are used by some pathogens to degrade phenols synthesised by host plants as inhibitors. This may stimulate the development of parasites (Bell, Treshow 2002). A mass occurrences of powdery mildews in Toruń has also been observed by Hołownia and Kostrzewska (1991), who believe such phenomena could be caused by air pollution. An epiphytositic occurrence of

E. alphitoides in areas strongly influenced by pollution has also been observed by Boczoń (1998) and Domański et al. (1987). Divergent results of research into the occurrence of *E. alphitoides* is explained by Domański (1976). He attributes epiphytoses to a specific system of ecological factors that favourably influence the development as well as aggressiveness and pathogenecity of the disease agent. A set of factors co-interacting in different combinations most probably occurred in each of the above cases and may have influenced divergent study results. This was shown in a study by Grzebyta et al. (2005), who analysed the influence of high temperature and fluoride on the development of *E. alphitodes* on *Q. robur*: higher infection of leaves in oaks growing in an area containing fluoride than in a clean area was recorded in previous observations. This indicated a low sensitivity of the pathogen to pollution with fluoride compounds. More detailed examinations, however, showed that fluoride which acted synergistically with high temperature caused the opposite effect and led to low infection of leaves.

Erysiphe hypophylla, which mostly colonised the lower leaf surface similarly to *E. alphitoides*, developed in all the distance zones. Its strongest development was observed at sites up to 300 m and at control sites. Interestingly, the species co-occurred on the same leaves and the percentage of the occurrence of this type was high in the distance zones in the three study years. Information on the occurrence of biology of *E. hypophylla* is scarce in the available literature. It was recorded in the Białowieża National Park by Majewski (1971) and at a few localities in Poland by Sałata & Majewski (1976) and Dynowska et al. (1999). The species seems to be common and the present study shows that it often occurs along with *E. alphitoides*. However, the presence of the latter is recorded in many Polish studies (Adamska et al. 1999; Czerniawska 2001; Kalinowska-Kucharska, Kadłubowska 1993; Mułenko, Wojdyło 2002). These studies are conducted mostly in environments similar to natural ones where the degree of anthropogenecity is low. These results, however, cannot be used to define the occurrence of *E. hypophylla* in urbanised and natural areas. Divergent opinions on the separate position of the two parasites and their treatment as one species, *Erysiphe* (*Microsphaera*) *alphitoides*, may have also contributed to this.

Weather conditions also influenced the development of the two species in the study period. *Erysiphales* can infect plants in a broad range of temperatures and humidity. Due to a considerable amount of water in the cell, spore germination in the majority of the representatives of the order takes places even in low relative air humidity. The highest degree of infection in all the species analysed in the study was observed in 2002 when the vegetative period was very warm and dry. July, August and September were hot while precipitation between May and September was lower than usual. Similar results were obtained by Durska (1974), who recorded an increased development of *Erysiphales* during a dry and hot summer.

Parasites aim to close the developmental cycle in order to produce structures having the best possible resistance genes that condition the survival of a species. Therefore, a strategy aimed at increasing and improving reproduction is a characteristic trait of parasites. Organisms whose features are better adapted to their environments achieve a greater reproduction success. The sexual process may be disturbed in unfavourable conditions, such as pollution, which may result in the absence of or a reduction in the number of ascomata or degeneration. This was observed in *Lophodermium pinastrii* by Grzywacz (1976) as well as by Kowalski and

Budnik (1976). The parasite could not reach a full developmental cycle due to high concentrations of toxic compounds and altered chemism of infected needles, and did not produce ascomata.

A full developmental cycle of the two powdery mildews that ended in the production of chasmothecia, also called cleistothecia, characteristic of this fungal group, was observed in the present study. Chasmothecia in the order *Erysiphales* are sclerotia and their initiation occurs in unfavourable environmental conditions according to some researchers (Dynowska 1996a; Füzi 2001). Literature data show that the majority of powdery mildews enter the reproduction stage towards the end of the vegetative period, between August and November (Füzi 2001; Majewski 1971; Mmbaga 2002; Sałata 1985; Turnau, Czerwonka 1986). Present results corresponds with literature reports. Young chasmothecia in *Erysiphe alphitoides* and *Erysiphe hypophylla* were observed at most sites at the beginning of August. They began to mature in the second half of August and mature chasmothecia were recorded in mid September. Similar results were obtained by Kadłubowska, Kalinowska-Kucharska (1989) and Minkievič et al. (1993). However, the parasites did not always enter the generative stage. The fact that the *Oidium* form was recorded towards the end of the study periods suggests that the sexual stage is not obligatory in the species and the parasite also overwinters in the anamorphic form. Majewski (1971) also observed that not all powdery mildews enter the teleomorphic stage in his studies in the Białowieża National Park.

Statistically significant differences in the number of chasmothecia between the zone influenced by automotive exhaust gases and the control zone were observed in *Erysiphe alphitoides*. Parasites formed chasmothecia more numerously at a distance up to 50 m away from transport routes and their smallest number was recorded >300 m. This may result from the differences in the degree of infection of the host plant. A similar opinion was expressed by Füzi (2001), who observed a strict correlation between the number of chasmothecia and the degree of infection: both variables were mutually dependent. Mmbaga (2002) disagrees and reports that neither the degree of infection of the host (leaves with a low infection degree often contained a high number of chasmothecia) nor the plant age influences the number of chasmothecia. Significant disturbances in chasmothecium development at sites located by main transport routes were not observed in the two powdery mildews.

It is interesting that differences in appendage development were observed between the two mildews infecting *Q. robur*. A considerably greater number of chasmothecia without appendages (0 developmental stage) and having appendages not fully developed (I developmental stage) were recorded in *E. alphitoides*. Chasmothecia having appendages in I and II developmental stage dominated in *E. hypophylla*. The lower leaf surface may have been more favourable for the development of chasmothecia. This is reflected in the maturity of asci and spores. A high participation of chasmothecia without developed asci and spores was recorded in *E. alphitoides* while chasmothecia with asci containing spores constituted a considerably higher percentage in *E. hypophylla*.

Untypical chasmothecia whose size was considerably smaller and which did not have developed asci and spores but had fully developed appendages and chasmothecia with untypical appendages were observed in *Erysiphe alphitoides*. These chasmothecia were recorded in the zone up to 50 m in 2002. Empty chasmothecia have been described in *Epichlöe typhina* in athropogenic populations of *Pucciniella distans* and attributed to disturbances in the genetic control of the developmental cycle of *E. typhina* caused by an environmental factor (Falińska 2002). Degenerated chasmothecia of a considerably smaller size were observed in *Lophodermium pinastrii* in the zone of the strongest influence of pollution by Benben, Sierota (1976) and Grzywacz (1976). Empty chasmothecia as well as untypical appendages in polluted sites recorded in the present study may indicate developmental disturbances. However, such chasmothecia constituted a very low percentage. Their occurrence may thus be caused natural disturbances resulting from the formation of a great number of chasmothecia or the influence of higher temperatures as suggested by Moore-Landecker (1992).

Ecological research into the parasites of the order *Erysiphales* conducted in the urban environment does not unambiguously show the influence of individual factors on the development of the fungi examined in this study. However, the two species have a broad ecological amplitude. This is connected with adaptation processes conditioned by genetic mechanisms that are activated at times of greater and more long-term amplitudes of environmental factors that are characteristic of urbinocenoses (Stearns 1992).

CONCLUSION

The absence of major disturbances in the life cycles of *E. alphitoides* and *E. hypophylla*, the occurrence of all developmental stages with the predominance of the perfect stage and a high rate of infection of the host plants in the vicinity of transport routes show a high adaptation degree of the two fungi in the environment strongly affected by anthropopressure.

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Rozwój *Erysiphe alphitoides* i *E. hypophylla* w środowisku miejskim

Streszczenie

Celem pracy było prześledzenie poszczególnych etapów cyklu rozwojowego *Erysiphe alphitoides* i *E. hypophylla* oraz ocena występowania tego pasożyta na *Quercus robur* w warunkach poddanych silnej antropopresji. Obserwacje prowadzono na terenie miasta Olsztyna i okolic podczas trzech sezonów badawczych. Stanowiska w liczbie 63 zlokalizowane były wzdłuż głów-

nych szlaków komunikacyjnych w odległościach do 50 m, do 100 m, do 300 m oraz >300 m (kontrolne). Materiał badawczy stanowiły losowo zebrane liście z rośliny żywicielskiej.

We wszystkich latach badań *E. alphitoides* i *E. hypophylla* wystąpiły na *Q. robur* z dużym nasileniem. Odnotowano stosunkowo wysoki udział wspólnego występowania obydwu gatunków na tych samych liściach.

Stwierdzono różnice w średnim stopniu porażenia rośliny żywicielskiej w zależności od odległości od szlaków komunikacyjnych. Wyniki te były istotne statystycznie. Nie odnotowano zakłóceń w rozwoju analizowanych pasożytów-obserwowano stadia anamorficzne i teleomorficzne niezależnie od odległości. U *Erysiphe alphitoides*, odnotowano istotne statystycznie różnice w liczbie chasmotecjów pomiędzy strefą znajdującą się pod wpływem spalin samochodowych a strefą kontrolną. Interesujące wydaje się zaobserwowanie różnicy w rozwoju przyczepek. U *E. alphitoides* odnotowano znacznie więcej chasmotecjów bez przyczepek oraz z przyczepkami nie w pełni rozwiniętymi. Natomiast u *E. hypophylla* dominowały owocniki z przyczepkami w pełni rozwiniętymi. Ma to odzwierciedlenie w dojrzałości worków i zarodników.

U *E. alphitoides* stwierdzono wysoki udział owocników bez wykształconych worków i zarodników, podczas gdy u *E. hypophylla* znacznie większy procent stanowiły chasmotecja z workami wypełnionymi zarodnikami.

W strefie do 50 m u *Erysiphe alphitoides* zarejestrowano owocniki nietypowe, o znacznie mniejszych wymiarach bez wykształconych worków i zarodników, ale z całkowicie wykształconymi przyczepkami a także owocniki ze zniekształconymi przyczepkami.