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REVIEW in MYCOLOGY

Scientific Research Conducted at the Department of Mycology, University of Warmia and Mazury in Olsztyn

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Abstract

This paper presents a review of the research conducted by the staff of the Department of Mycology at UWM, Olsztyn since its establishment to the present. This unit was established and has been headed for over 20 years by Prof. Maria Dynowska. Since 2004, the Department has been conducting extensive mycological research, which is reflected in the dynamic growth of specialist staff involved in teaching activities and popularizing scientific research. Owing to the particular care of Prof. Dynowska, and maintenance of the principal interdisciplinary character of the research, the Department has been occupying a significant position in mycology in Poland recently. This paper attempts to provide a summary of the major scientific accomplishments of the team headed by Prof. Dynowska.

Keywords

potentially pathogenic fungi; aquatic ecosystem; school environment; urban environment; lichenological studies; plant parasites; bioindication

1. Introduction

The Department of Mycology of the University of Warmia and Mazury in Olsztyn was established on April 1, 2004. It was the product of the transformation of the Department of Mycology established in 1996 from the Department of Botany at the Institute of Biology and Environmental Protection, Faculty of Mathematics and Life Sciences of the Teachers College in Olsztyn.

Prof. dr. hab. Maria Dynowska was appointed Head of the Department in 1996. Initially, the research conducted at the department covered the issues of botany and plant physiology in a broad sense. Over time, the subject matter evolved toward pure mycology. In consequence, mycology was isolated from botany, and the teaching offer was expanded to include obligatory and elective mycological subjects (general and systematic mycology, lichenology, phytopathology, medical mycology, hydromycology, mycosociology, mycoindication, mycological diagnostics, applied mycology, etc.), whose status in the syllabus was adjusted to that of the major subjects (biology, biotechnology, microbiology, and nursing), and two research teams were appointed: The Ecology and Environment Monitoring Team and the Applied Mycology Team. The teams included mycologists and botanists, who prepared an assessment of the environment against which fungi were analyzed (saprotrophs, phytopathogens, zoopathogens, anthropopathogens, and lichenized fungi). Upon the establishment of the University of Warmia and Mazury in Olsztyn, the scope of research was largely modified, and some of the staff of the Department of Mycology were transferred to other University units. Owing to the initiative of Prof. Dynowska, new research teams, viz., the Fungi and Lichens Monitoring Team and the Medicinal and Applied Mycology Team, were appointed. These

teams have operated throughout the history of the Department. The research was interdisciplinary, and covered issues in mycology, microbiology, phytopathology, lichenology, and ecology, with emphasis on the diversity of fungi in macro- and microenvironments and the significance of fungi in bioindication (in a broad sense of the term). It was conducted based on two multitask subjects:

- Biological and ecological factors that affect fungi diversity and expansiveness;
- Fungi and lichens against natural plant communities.

By presenting the findings of the research conducted at the Department via this review paper, we wish to express our gratitude to Prof. Dynowska for her scientific supervision, care for the development of our research, and continuous support for our cooperation with various research centers. Each chapter was prepared by Prof. Dynowska's protégés, and presents diverse specialties of mycology.

2. Potentially Pathogenic Fungi Isolated from Waters and Their Application in Bioindication

Research on the presence of fungi in various types of waters has been conducted around the world since the 1960s. It has dealt with mycological environmental monitoring in general terms (Cooke, 1965; Laundon, 1972), and resulted in the development of standards of cleanliness in the USA and Canada, with fungi as bioindicators of pollution in household, industrial, agricultural, and touristic wastewater (American Society for Testing and Materials, 2005). Hydromycological research in Poland has been conducted with regard to the number, species diversity, and ecophysiology of fungi in waters of different trophic states, with attention also being paid to potentially pathogenic species (Dynowska, 1995; Kurnatowski et al., 2007). Continuous mycological monitoring of waters in north-east Poland was initiated in the 1990s by Prof. Dynowska (Dynowska, 1993b, 1993d, 1995, 1997), and it has been continued by her research team. The scope of research has been expanded to include water bodies in the north-west (Biedunkiewicz et al., 2007) and central parts of Poland (Biedunkiewicz & Ozimek, 2009; Ozimek & Biedunkiewicz, 2008). The findings of the research not only explain the role of microfungi in the trophic and energetic loops in water bodies, but also help in identifying specific fungi with indicative properties, which can be used in a multiaspect assessment of waters, especially regarding their sanitary and epidemiological quality (Biedunkiewicz, 2011b; Dynowska, 1995, 1997).

The hydromycological research focused on microfungi (potential human pathogens) present in different types of waters, their numbers and taxonomic differentiation during all seasons of the year, and those with interesting epidemiological and bioindicative features. Analyses were performed for tap and potable bottled water (Biedunkiewicz et al., 2014; Biedunkiewicz & Schulz, 2012), and water from places used for recreation, including municipal lakes and indoor swimming pools (Biedunkiewicz, 2007a; Biedunkiewicz & Górska, 2016; Biedunkiewicz et al., 2007), as well as fountains situated in parks and at squares used by residents for leisure (Biedunkiewicz, 2009). It was shown that, in the immediate vicinity of bathing sites, the existing littoral zone retained yeasts (potential human pathogens) on the phyllosphere surface. Long-term observations in one of the lakes in Olsztyn have shown the presence of the same microfungi species in both habitats in 60% of cases. The presence of many potentially pathogenic yeast species in the lake littoral zone shows the huge impact of humans on the hydrosphere microbiological quality (Biedunkiewicz et al., 2020).

The frequent presence of microfungi (including *Candida albicans*, *C. tropicalis*, *C. guilliermondii*, and *C. krusei*) in waters used, in a variety of ways, by humans for consumption or recreation has prompted observations of some ecophysiological features of these fungi, such as the multiplication rate, enzymatic activity, and pseudomycelium growth capability in controlled laboratory conditions, with the use of modified media based on filtered water acquired from the natural environment, and a photoperiod appropriate for the given environmental and daily temperature fluctuations. One of the parameters – enzymatic activity – was tested before and

after culturing four of the aforementioned fungi species in the laboratory. The results showed that the microfungi retained a high physiological activity. Hence, they should be entered in the list of pathogens whose presence in utility waters, including thermal and recreational waters, disqualifies them from public use. One cannot exclude the role of water as a habitat for potential pathogens, because depending on the type, amount, and availability of organic matter, numerous fungi species find favorable trophic conditions for development, which have a direct impact on the metabolism rate (Biedunkiewicz, 2015). The study was conducted as part of a research grant from the Ministry of Science/National Centre for Science, entitled “Próba zastosowania grzybów potencjalnie chorobotwórczych dla człowieka w standardowych metodach oceny czystości wód powierzchniowych [An attempt to use fungi – potential human pathogens – in standard methods of surface water cleanliness evaluation].”

As a permanent component of inland water microbiota, fungi were present in all the types of water under study. However, an increase in their number depended on the degree of anthropopressure and eutrophication. The eutrophic lakes were found to contain numerous, frequently repetitive species of potential pathogens or saprophytes; some fungi that had not been reported in the literature on hydromycology were also isolated, viz., *Candida kruisii* and *Dipodascus armillariae* (Biedunkiewicz & Baranowska, 2011).

The research conducted at the Department to date has demonstrated the diversity of microfungi in waters and the increase in their numbers in proportion to the pace and intensity of anthropogenic changes. This applies mainly to fungi that prefer strongly eutrophicated waters that are rich in organic matter of various origins. These fungi include ascomycetes and basidiomycetes, as well as some molds. It must be emphasized that the majority of the species of these microfungi groups isolated from waters are potential aetiological agents of surface and organ mycoses, and their abundant proliferation in the waters under study may pose a serious epidemic threat. Owing to this, a relatively complete picture of the water quality can be shown with respect to the sanitary and epidemic status, taking into account fungi with high pathogenic potential (Biedunkiewicz et al., 2013).

The majority of fungi isolated from water are opportunistic, and can settle on the human skin and mucous membrane, most frequently in the respiratory and alimentary tracts (Biedunkiewicz, 2011a). This may be temporary settlement or colonization, which does not produce symptoms in carriers, while people with impaired immunity acquire mycotic infection. The abundance of fungi that colonize various sections of the respiratory tract was demonstrated in a multiyear study conducted at Samodzielny Publiczny Zespół Gruzlicy i Chorób Płuc [Independent Public Tuberculosis and Pulmonary Disease Unit] in Olsztyn, which was the starting point for the hydromycological studies described herein. These studies produced surprising findings and provided the basis for the doctoral thesis entitled *Dynamika mikoflory układu oddechowego człowieka* [Mycoflora dynamics in the human respiratory tract], with Prof. Dynowska as a scientific supervisor. The analyses revealed the presence of 29 species of eight genera, viz., *Candida*, *Geotrichum*, *Saccharomyces*, *Saccharomyopsis*, *Schizosaccharomyces*, *Torulopsis*, *Trichosporon*, and *Aspergillus*. Many of these fungi have been isolated in the hospital environment, indicating that the same species present in the human environment can appear in this ontosphere under favorable conditions, i.e., immunosuppression and dysbacteriosis (Biedunkiewicz, 2001; Dynowska et al., 2002, 2013). These findings suggested the need for mycological analyses in healthy individuals, i.e., students of biology and veterinary medicine (Biedunkiewicz, 2007b, 2011a). The results confirmed the carriage of 15 species of potentially pathogenic fungi of seven genera in nearly 80% of the tested individuals. Combined with the results of clinical observations, they directed the researchers' attention to natural reservoirs of fungi in immediate human surroundings.

The list of fungi that have been isolated from waters to date is long, and contains 185 species. Each aquatic ecosystem under study was found to contain *C. albicans*, although it was not always a dominant species. It is particularly important in the context of the relationship of the species with the ontospheres of humans and

other vertebrates, which poses a potential violation of the biological balance, and breakdown of body immunity. The presence of *C. albicans* in polluted waters suggested its usability as an indicator of cleanliness and sanitary security, especially since this species survives longer in marine and fresh waters than *Escherichia coli* (Biedunkiewicz, 2015; Dynowska, 1995). Microfungi also survive at low temperatures. The psychrosphere is not a habitat barrier for them. This has been confirmed via mycological analyses of ice and snow cornices (Biedunkiewicz & Ejdys, 2011; Ejdys, Biedunkiewicz, et al., 2014). All species proved to be psychrotolerant, and they retained high viability following incubation at 37 °C. These research findings provide grounds for regarding ice and snow cornices as natural and temporary air filters and specific vectors of potential pathogens, which pose a real epidemic threat in the environment.

The hydrosphere is one of the greatest fungi reservoirs with a diverse taxonomic and trophic status. In addition to saprotrophs with dormant pathogenic potential, species regarded as human and animal pathogens have been isolated from water bodies. Quantitative and qualitative assessments of isolated microfungi are very important in comprehensive mycological studies of various aquatic ecosystems and utility waters. An analysis of the prevalence of their occurrence in recreationally used waters, especially in summer, is imperative. There may be no officially published epidemiological data concerning the correlations between microfungi concentrations in waters and infections in humans who have contacts with them; however, the occurrence of fungi in waters suggests such a possibility, and also indicates that a water reservoir can be a serious source of mycoinfections. Current knowledge of the ecophysiology of potentially pathogenic fungi and long-term studies indicate that waters are important links in the mycoses epidemic chain in the biosphere, especially if they contain fungi from household waste, asymptomatic carriers, or people with mycoses (Dynowska, 1995). Therefore, important achievements in hydromycological research include the development of a procedure for the isolation, counting, and identification of potentially pathogenic fungi, which can be used by laboratories in routine sanitary and epidemiological testing of waters used for various purposes. This was incorporated, together with six key publications (Biedunkiewicz, 2011b, 2015; Biedunkiewicz & Baranowska, 2011; Biedunkiewicz & Ejdys, 2011; Biedunkiewicz & Góralaska, 2016; Biedunkiewicz & Ozimek, 2009), in the scientific accomplishment entitled “Możliwości wykorzystania wybranych mikrogrzybów do oceny sanitarno-epidemiologicznej wód na tle różnorodności i fenologii gatunków potencjalnie chorobotwórczych dla człowieka [Use of selected microfungi in sanitary and epidemiological assessment of water against the background of diversity and phenology of potential human pathogens],” which was the basis for conferring the scientific degree of doctor habilitated in biological sciences, microbiology specialty.

The research conducted to date at the Department has been devoted to the taxonomy, quantitative aspects, and phenology of isolated fungi against the background of natural and anthropogenic factors in aquatic environments. The research findings have been published in nearly 110 scientific papers, showing a relatively complete picture of water quality with respect to its sanitary and epidemic status, taking into account fungi with high pathogenic potential (Dynowska & Biedunkiewicz, 2013).

3. Circulation of Potentially Pathogenic Fungi in a School Environment

The research conducted by Prof. Dynowska on potentially pathogenic fungi in the biosphere was expanded in the 2000s to include analyses of their occurrence in organ ontocenoses in healthy children and adolescents in rural and urban environments. The research conducted for the doctoral thesis entitled *Analiza mikologiczna wybranych ontocenozy dzieci w wieku 6–15 lat* [Mycological analysis of selected ontocenoses in children aged 6–15 years] (Ejdys, 2002) was used as the starting point. It showed that schools were very interesting and poorly explored objects of mycological research, especially with respect to the factors affecting the occurrence of fungi in children and adolescents, as well as their surroundings. The

results also encouraged researchers to plan studies of importance to epidemiology, concerning multidirectional and multilevel analyses of the transmission of these fungi.

The greatest attention in pediatric mycology literature is devoted to children in risk groups. They include children with diabetes and cancers undergoing intensive chemotherapy, and immunosuppression and antibiotic therapy, or those being treated orthodontically. Few papers have been published on mycobiota in children and adolescents regarded as healthy. The first/pilot study was conducted in 1997 on 70 children aged 0–9 and 10–15 years (Dynowska & Ejdys, 2000), and was aimed at determining interindividual transmissibility within same-age groups. Forty-five isolates of eight fungi species were obtained. These species were present in 39% of the population under study, and they occurred in children more frequently than in adolescents. The phenological differences in the prevalence of fungi in the children under study were emphasized. Unlike in adults (Dynowska, 1993a), fungi were found twice more frequently in children's ontocenoses in spring than in fall. Obtaining multispecies isolates was an important outcome of this research. Considering the findings and the epidemiological importance of the artificially created population of children who stay as a group within a limited area of a school building for up to 9 hours a day, it was decided to expand the school environment research. Over three subsequent years, 270 generally healthy children aged 6–15 years and living in the Voivodship of Warmia and Mazury were examined (Ejdys, 2001, 2003a, 2003b). The research aimed to evaluate the relationship between the occurrence of fungi in children's oral cavities and noses, and the living environment (town–village), gender, age, diet, prior diseases, antibiotic therapy, and contacts with the hospital environment, as well as identify children at risk of mycotic infections. Fungi were found in 36.3% of the population tested, which was a figure comparable to that in preliminary examinations; 168 isolates of 13 yeast species were obtained. *Candida albicans* – the main aetiological agent in the majority of human mycoses – was a dominant species. The presence of *C. glabrata* and *C. krusei*, whose increasing number of strains demonstrated resistance to main antimycotics, and the relatively frequent occurrence of *Trichosporon beigelii*, *Saccharomycopsis capsularis*, and *Saccharomyces* spp. – fungi of growing expansiveness and enzymatic activity – was a cause for concern. Regardless of the season, the greatest fluctuations in the mycocenoses diversity and count were observed in the oral cavity and nose, which were the organs in direct contact with the contaminants in the environment and the many potentially pathogenic fungi transmission agents.

The threat of a mycotic infection in the majority of the tested population increased after antibiotic therapy, regardless of the season of the year or the age. This mainly concerned the most stable ontocenosis of the pharynx. Children living in villages, who had been ill immediately before the material was collected, were at the greatest risk of having fungi in any of the ontocenoses under study. This is a consequence of, among other things, more frequent contact of children in a rural environment with natural reservoirs rich in fungi (Ejdys & Dynowska, 2004).

Since the differences between the prevalence of fungi in girls and boys living in villages and those living in towns may have been caused by physiological, rather than environmental factors, a study aimed at determining the effect of gender on the frequency of fungal infections in children of school age was conducted (Ejdys, 2008a). In spring and fall, tests were performed on 238 girls and 206 boys (regarded as healthy) of the following ages: 6–9, 10–15, and 16–18 years. Although the prevalence of fungi was similar in both genders (♀32.7% and ♂30.58%), the species spectrum and occurrence of individual fungi species differed significantly among the tested groups. The occurrence of *C. albicans* in girls decreased compared to that of *C. tropicalis* and *C. dubliniensis*. An analysis of seasonal fungi occurrence, depending on the specificity of the tested ontocenoses and children's age, showed that each gender is susceptible to infection by a different complex of factors, with a correlation with age being observed for girls.

Infections of potential fungal pathogens in generally healthy children typically end with the elimination of the fungi from the children's bodies. According to the findings of these researchers' studies, it happens in approx. 65% cases (Ejdys, 2003a).

In other cases, commensalism or colonization is suspected, with possible transition to carriage. A single test of a child without any disease symptoms does not provide a basis for a clear identification of the relationship between the child's body and a potential fungal pathogen. An inconclusive interpretation of the results of a single test of children led the researcher to conduct a distance control, whose aim was to differentiate fungal infections in healthy children into temporary and permanent, and determine the factors that predispose children to temporary fungal infections (commensalism and colonization) and carriage (Ejdys, 2006).

Tests were conducted on smears taken twice within a 5-year span during the same season of the year from the oral cavities, pharynxes, and noses of 34 children aged 7 and 12 years. Fungi were found in seven children in the first test and in nine children in the second test, and the fungi incidence was 20.6% and 26.5%, respectively, with fungi found only in three girls (8.8%) in both tests. Single-focus infections were the most frequent. Fungi were isolated from the ontocenosis of the oral cavity and the pharynx of one child in each test. Differences in the ontocenoses diversity were observed in both tests. It was concluded that the children in whom fungi were found twice could be carriers. The physiological status of cohabitation with fungi in children with a single infection cannot be determined. However, all positive results must be treated very seriously in the context of potential future mycosis caused by yeasts (Ejdys, 2006).

Initially, the research conducted in the school environment focused on fungal infections in children (Dynowska & Ejdys, 2000; Ejdys, 2003a), to identify individuals particularly susceptible to infections (Ejdys, 2003b), and factors that favor colonization of organ ontocenoses by various yeast species (Ejdys, 2008a; Ejdys & Dynowska, 2004). However, in addition to individuals potentially susceptible to infections, there are other sources, ways, or vectors of fungal infections in the school environment.

Air is an important vector of fungi transmission between organisms (Dynowska & Ejdys, 1999). Fungal diaspores account for the majority of bioaerosols in atmospheric air. Spores of yeast-like fungi, which are heavier than those of mold fungi, occur in the bottom part of the air column. This is the height at which there are two main gateways of infection in primary school children: the mouth and nose. Therefore, a high density of fungi in the air may pose a serious threat to the health of children who breathe in such air. The presence of the aforementioned fungi species in the air within and around school buildings was examined in May (heating on) and November (heating off), and the fungal features that help them survive in the school environment were analyzed (Ejdys, 2008b; 2009b; Ejdys et al., 2009). The air in school buildings, of the right humidity and temperature, was found to contain 28 species of 15 genera of yeast-like fungi. The atmospheric air was found to contain 20 species of 14 genera. *Kluyveromyces lactis* dominated in the indoor air, whereas *Yarrowia lipolytica*, which is the species with the highest symbiotic capability, dominated in the air outdoors. A comparison of the habitats under study showed, from an epidemiological perspective, that schoolrooms have greater potential to cause infections than the air outdoors. The worst yeast species spectrum was observed in intensively used rooms with limited access to light and oxygen; hence, they are the greatest threat to the health of children and school personnel.

Nonfermenting isolates and those that did not produce pigments dominated in heated rooms, whereas fermenting fungi and those capable of producing pigments dominated when heating was off (Ejdys, Dynowska, et al., 2014). The ability to produce carotenoid pigments is clearly promoted in indoor yeast. It may be associated with the addition of intermediate stages of carotenoid biosynthesis or an excess of oxidative compounds. Two-thirds of the isolates did not require any vitamins for their growth. Five isolates of four species exhibited acid-formation properties: *Dekkera anomala*, *D. bruxellensis*, *Y. lipolytica*, and *C. tropicalis* (syn. *C. citrica*). Owing to acid production, the fungi could dissolve inorganic compounds in building structures and release essential micronutrients. These findings suggested that some yeasts inhabit rooms as their main environment, whereas some live there temporarily because the human body is their main habitat (Ejdys, 2008b).

Determination of the survival factors for the fungi outside the human ontosphere was regarded as particularly important (Ejdys, 2009a; Ejdys & Raszteborska, 2010). Isolates of *C. albicans*, *C. dubliniensis*, and *C. tropicalis* obtained from human organ ontocenoses were used in the research. The strains were kept on dry glasses, with only access to the aerosol in the laboratory room, and their viability and survival rate was assessed every two weeks. A 100% death rate was not noted for any of the isolates during the 22-week observation; this proves high viability and suggests the need for improved hygiene in schoolrooms.

A 4-year mycological monitoring of school rooms demonstrated that yeasts account for a third of the mycobiota in the air and on walls; a total of 772 fungal isolates of 151 species and 48 genera were obtained in the test, with 70 sterile isolates. Fungi of the genera *Aspergillus* (36 species), *Penicillium* (26), and *Candida* (14 + two anamorphs) were the most frequent. *Aspergillus fumigatus* was the dominant species (126 isolates). This fungus is a permanent component of mycobiota in schools/public buildings in north-east Poland. Its count in rooms should be monitored continuously; this applies particularly to thermophilic isolates, owing to their environmental plasticity and potential pathogenicity (Ejdys et al., 2013).

Such a broad spectrum of fungi species has not yet been observed in studies of indoor bioaerosols in this part of Europe (Ejdys, 2007a, 2007b; Ejdys & Biedunkiewicz, 2011; Ejdys et al., 2013). It seems that it is largely a consequence of the adopted research methodology (Ejdys, 2011; Ejdys et al., 2015). Simultaneous application of different incubation temperatures (25, 37, and 40 °C) allowed for the broadening of the known mycobiota spectrum in schoolrooms. Sterile isolates (approx. 10%) pose an as yet undiscovered potential threat to users of the building itself, related to pathogenicity, especially allergenicity and toxin production potential, since all the 33 randomly selected fungi isolates obtained from the rooms demonstrated toxin production potential (Ejdys et al., 2013).

The research on the school environment conducted at the Department of Mycology, UWM in Olsztyn resulted in the publication of 34 papers and over 40 research communications. In addition to the original papers, others devoted to research methodology, describing the methods and techniques to collect and diagnose study material more effectively, were published.

4. Lichenological Research

The beginnings of lichenological studies in Warmia and Mazury date back to the late nineteenth century. The material collected in the region (the former German province of East Prussia) by Ohlert (1870) provided data that are still valuable as they enable researchers to assess the changes that have taken place in lichen biota over 150 years (Cieśliński, 2003). After nearly 100 years, research on lichens was started at the Department of Botany of the Academy of Agriculture and Technology in Olsztyn (currently Department of Botany, UWM in Olsztyn). The research was conducted by Hutorowicz (e.g., Hutorowicz, 1957, 1963), and subsequently, Zalewska and her team (e.g., Kukwa, Łubek, et al., 2012; Szydłowska et al., 2013; Szymczyk et al., 2014, 2015; Zalewska, 1998, 2012; Zalewska et al., 2004a, 2004b).

Lichenological research at the Department of Mycology of the UWM in Olsztyn was started in 1997 (Kubiak, 1999), initially at the Botany Department of the Teachers College in Olsztyn. One of the researchers involved was Professor Stanisław Cieśliński, an eminent Polish lichenologist. Affiliated to a scientific center in Kielce, he worked for a brief period (1993–1994) at the Botany Department of the Teachers College in Olsztyn as a researcher and teacher. During that time, he conducted extensive field research, the results of which were published in the *Atlas rozmieszczenia porostów (Lichenes) w Polsce północno-wschodniej* [Distribution atlas of Lichens (Lichenes) in north-eastern Poland] (Cieśliński, 2003). The introduction to the atlas states that “the good condition of the lichen biota [in the north-east of Poland] and preservation of biocenotic associations with lichens allows tracing and describing processes and relationships which – due to the anthropogenic impact, its pace and range – is not possible elsewhere in Poland.” The space for such lichenological research was noted by Prof. Dynowska, and she initiated the

related actions soon after taking over the position of Head of the Department of Mycology of the Teachers College in Olsztyn. Dariusz Kubiak was appointed head of this field of research at the Department. The initial period of the research was the time when a new approach to taxonomic studies was becoming increasingly popular among lichenologists. It involved a broader use of biochemical features in species identification (Guzow-Krzemińska & Kukwa, 2013; Kubiak & Kukwa, 2011), and was made possible by the development of techniques and procedures of chromatographic separation of secondary metabolites of lichenized fungi, the so-called “lichen acids” (Tønsberg, 1992). Owing to cooperation with other research centers, particularly with the Department of Plant Taxonomy and Nature Conservation of the University of Gdańsk (currently: Laboratory of Lichenology and Experimental Mycology), a laboratory of planar thin-layer chromatography (TLC) was established at the Department of Microbiology and Mycology; this proved essential in the first research projects carried out at the Department.

The north-east of Poland, as an area of lichenological research, is a very interesting region from a scientific perspective (Cieśliński, 2003); however, it is also a highly demanding region because of the abundance of lichen biota and the number of lichen species. The initial research conducted at the Department focused on exploring the taxonomic diversity, ecology, and distribution of lichen biota in the urban areas of Olsztyn (Kubiak, 2005). The research findings, analyzed from a bioindicative perspective, provided the basis for the doctoral thesis written by Dariusz Kubiak, entitled *Porosty Olsztyna na tle antropogenicznych przekształceń środowiska* [Lichens in Olsztyn against the background of anthropogenic transformations], with Prof. Dynowska as the scientific supervisor. The research, conducted for many years, documented the occurrence of over 300 lichen species within the city borders, which is a phenomenon on a global scale. (Kubiak, 2005; Kubiak, Sucharzewska, & Ejdyś, 2016).

The scope of the research was gradually expanded to include other areas poorly explored from a lichenological perspective, especially those regarded of key importance to lichen diversity in the region (Kubiak, 2011b; Kubiak, Biedunkiewicz, & Ejdyś, 2015; Kubiak, Biedunkiewicz, & Koźniewski, 2015; Kubiak et al., 2014, 2017, 2019; Kubiak & Sucharzewska, 2018). In some cases, the study findings proved so interesting that they were the basis for the decision to initiate special protective measures (Kubiak, Biedunkiewicz, & Ejdyś, 2015; Kubiak et al., 2014; Kubiak & Sucharzewska, 2013, 2018) or, in the case of protected sites, to expand the scope of protection (Kubiak et al., 2017).

The nature and scope of the research, as well as the specificity of the region, which stands out against other parts of the country with respect to preservation of biodiversity, resulted in the accumulation of a large amount of data on the distribution and habitat preferences of many lichen species. The data concerning a group of sterile crustose lichens, which have not been well-explored in Poland, are of particular value. Such data made it possible to make the first, relatively reliable assessment of the current status of the species and the danger to them (Kubiak, 2010, 2011a; Kubiak & Łubek, 2016; Kubiak & Sucharzewska, 2014; Kubiak & Westberg, 2011; Kubiak & Zalewska, 2009). The discovery of new taxa of the lichen biota in Poland was one of the particularly valuable results of the research (Kubiak, 2003; Kubiak & Malicek, 2012; Kubiak & Westberg, 2011; Kubiak & Wilk, 2016; Kukwa & Kubiak, 2007); these include *Loxospora cristinae* Guzew-Krzemińska, Łubek, Kubiak & Kukwa (Guzew-Krzemińska et al., 2018). The relatively broad scope of the research was made possible by the fact that it was financed as part of the Ministry of Science/National Centre for Science research project entitled “Zróżnicowanie i środowiskowe uwarunkowania licheni bioty lasów grądowych (*Carpinion betuli*) Pojezierza Mazurskiego [Diversity and environmental factors affecting the lichen biota in the oak-hornbeam forests (*Carpinion betuli*) in the Masurian Lakeland].”

At the same time, lichenological research was initiated in other parts of Poland. Among the more significant studies conducted at the Department of Mycology were those conducted in the Rogów forests (Kubiak & Szczepkowski, 2012), Mazovia (Kubiak, 2009, 2013; Kubiak, Biedunkiewicz, & Koźniewski, 2015; Kubiak

et al., 2010), and the south of the Wielkopolska region (Kubiak, 2008; Kubiak & Biedunkiewicz, 2015). The Department participated in many team projects whose aims included increasing the state of knowledge on lichen biota in selected regions of Poland (Fałtynowicz et al., 2015; Kukwa, Kowalewska, et al., 2012; Kukwa et al., 2008), Lithuania (Motiejūnaitė et al., 2012), and Latvia (Motiejūnaitė et al., 2016).

As the research at the Department of Microbiology and Mycology intensified, it became increasingly specific. The research activities at the Department focused, on the one hand, on issues related to lichen diversity, viz., methods of its assessment, principles, and ways of data interpretation (Kubiak, 2012), and on the other, on the usability of the research findings in activities aimed at the preservation or sustainable exploitation of natural resources (Kubiak, Osyczka, & Rola, 2016). Many papers note the potential role and importance of old trees in the conservation of lichen diversity in forests, particularly that of stenotopic forest lichens (Kubiak & Osyczka, 2017; Kubiak & Sucharzewska, 2012, 2018). As a specific summation of this stage of lichenological research conducted at the Department of Microbiology and Mycology, Dariusz Kubiak received the title of doctor habilitated of natural sciences, biological sciences discipline, based on the scientific achievement entitled “Naturalne i antropogeniczne uwarunkowania różnorodności porostów epifitycznych [Natural and anthropogenic factors affecting the diversity of epiphytic lichens].”

The current period of lichenological research at the Department of Microbiology and Mycology covers issues related to the identification of environmental factors that are potentially significant to species occurrence, and an assessment of their impact on the diversity of epiphytic lichens, on the site, habitat, and region/landscape scales. This research includes various types of forest communities as well as selected forms of the cultural agricultural landscape, which includes alleys of roadside trees (Kubiak & Osyczka, 2019, 2020; Osyczka & Kubiak, 2020). To understand and study the regularities of epiphytic lichen ecology, the body of environmental data, related both to the qualitative and quantitative diversity of lichen biota, as well as specific physicochemical parameters of their environment, is subjected to multidimensional statistical analyses and ordination techniques. The aim is to use this solid basis to create scientific grounds for practical measures to conserve natural resources and preserve the service that they perform for the ecosystem, which is particularly important and urgent in the face of newly emerging environmental threats.

The scientific achievements of the Department of Microbiology and Mycology in lichenological research include 70 original papers published during the past 20 years. In addition to the scientific papers, the Department has accumulated extensive reference material in the form of herbarium exhibits kept at the Department of Microbiology and Mycology (OLTC). A considerable number of sterile crustose lichens are some of the remarkable items in this collection. This material has significant scientific potential. It provided the basis for many published papers written by the Department staff, and is used by other researchers in taxonomic or phylogenetic studies.

5. Studies of Fungi – Parasites of Plants in the Urban Environment

Parasitic fungi are an important link in each biocenosis, and their presence in an environment depends mainly on the accessibility of host plants (Majewski, 1971). Finding a suitable host, which will ensure survival in different environmental structures, is the most important element of a parasite's life strategy. A close relationship between a parasite and its host, which has developed by coevolution, produces an inseparable biological system affected by environmental conditions (Mułenko, 1998). The high diversity of plant species, many of which have been introduced by humans, as well as the dynamic nature of the urban environment, make urbicenososis an interesting basis for analyzing the species diversity of parasites, studying their life strategies and the appearance of new species, and registering cases of native species being pushed out. Life strategies of parasites in natural conditions, under biocenotic homeostasis, are generally well defined and relatively stable. On the other hand, under anthropopressure, all types of

reactions are changed frequently by disturbing factors, affecting the number and prevalence of phytopathogens. In urbiceneses, it is often a complex of transport-generated pollution, which can be of strategic importance for the growth and development of ectoparasites, such as powdery mildew, that play an important role in adaptation. As a response to such considerations, Prof. Dynowska conducted long-term studies on diverse responses and the bioindicative potential of fungi of the order Erysiphales in the environment under strong anthropopressure (Dynowska, 1993c, 1994, 1996a, 1996b). The reconnaissance study conducted by the researcher for several years in north-east Poland showed that there are Erysiphales species that respond in a variety of ways to urban, particularly transport-generated, pollution. By analyzing the degree of infestation of host plants in different pollution zones, expressed by the diseases index, she identified susceptible and resistant fungi. In the case of the former, a high concentration of exhaust fumes was accompanied by a decrease in the disease index, an increase in the mycelium growth rate, and earlier maturation of the fruit body compared to those in nonurban areas. She identified the following species as susceptible: *Erysiphe alphitoides* (*Microsphaera alphitoides*; Braun, 1987), *E. cichoracearum*, *E. heraclei*, *Golovinomyces sordidus* (*E. sordida*), and *Podosphaera fusca* (*Sphaerotheca fusca*, *S. erigerontis-caadensis*; Braun, 1987). In contrast, she identified the following species as resistant: *Erysiphe berberidis* (*Microsphaera berberidis*; Braun, 1987), *E. depressa*, *E. palczewskii* (*Microsphaera palczewskii*; Braun, 1987), and *E. vanbruntiana* (*Microsphaera vanbruntiana*; Braun, 1987; Braun & Takamatsu, 2000).

Since there were large gaps concerning the ecology of parasitic fungi in urbiceneses in the Polish literature of the subject, the findings of Prof. Dynowska's research inspired other researchers to start detailed studies of their own. Their aim was to observe the responses of selected Erysiphales species in the urban environment, and determine the degree and scope of changes in their growth cycle. Interactions in the "parasite-host" system, developed as a result of environmental changes, can be additionally modified by the presence of hyperparasites, which is why the presence of the Erysiphales hyperparasite, *Ampelomyces quisqualis* (*Cicinobolus cesatii*), was also taken into account in the study (Sucharzewska & Dynowska, 2002). Seven Erysiphales species were covered by the study, considering the presence of host plants in the area under study: *E. alphitoides* and *E. hypophylla* on *Quercus robur*, *E. palczewskii* on *Caragana arborescens*, *G. sordidus* on *Plantago major*, *Podosphaera fusca* on *Taraxacum officinale*, and *Sawadaea tulasnei* on *Acer platanoides* L. (Sucharzewska, 2007).

Several research tasks were adopted in order to accomplish the objective:

- Comparison of the degree of infestation of host plants by selected Erysiphales fungi species at sites located at various distances from the transport routes in the city of Olsztyn.
- Analysis of the share of anamorphous and teleomorphous stages in the material under study as a potential adaptation index.
- Analysis of the adult stage – the time when chasmothecia appear, their number, and the development of rhizines, sacs, and spores – depending on the distance from transport routes.
- Assessment of the impact of *A. quisqualis* – an Erysiphales hyperparasite – and other fungi that settle the thallus Erysiphales species on the growth of the fungi under study, and the degree of infestation of host plants.

The study findings demonstrated a higher degree of host plant infestation by all the phytopathogens selected for the study at the sites located near the main transport routes, compared to that at the control points. This led to several conclusions. In the case of *E. alphitoides* and *E. hypophylla*, a high disease index of *Q. robur* at sites located in the zone of accumulation of transport-generated pollution was proof of the resistance of both species to this type of pollution. This applied especially to *E. alphitoides*, which, in the phytopathological literature, was regarded as a species sensitive to urban and industrial pollution (Sucharzewska, 2009). The disease index of *C. arborescens* and *T. officinale*, infested by *E. palczewskii*

and *P. fusca*, respectively, was affected indirectly, but significantly, by human activity. Impairment of plant health by systematic plant care treatment (lawn and hedge trimming) and toxic car exhaust probably disrupted the basic physiological processes in “plant–host” partnerships in the ecological systems under study. In consequence, the dynamic equilibrium state was disrupted for the benefit of the parasites, expressed by a high degree of infestation of host plants, of even 100% (Sucharzewska & Dynowska, 2005). The high disease index for *A. platanoides* infested by *S. tulasnei* on young maples, and the very low disease index or lack of parasites on mature trees, suggest that the growth of this mildew species depends mainly on the host plant age. Older trees were more resistant and healthier than young ones (Sucharzewska, 2010). *Erysiphe palczewskii* was a species that stood out as one that accomplished all its life functions in the urban environment. It was here that the highest infestation degree in the host plant compared to that in the other representatives was observed, in addition to the presence of a fungus growth stage at every site, the largest number of fruiting bodies, as well as a >90% share of mature fruiting bodies with fully developed rhizines, sacs, and spores (Sucharzewska & Dynowska, 2005). Hyperparasites were found on all the Erysiphales species under study, with the largest number of them belonging to the genus *Ampelomyces*. A strong presence of other genera was observed on the mycelium of *E. alphitoides* and *E. palczewskii* at sites near the main transport routes, e.g., *Alternaria*, *Aureobasidium*, *Cladosporium*, *Stemphylium*, and *Tripospermum*. They reduced the number of fruiting bodies of host fungi, which proved the weaker condition of Erysiphales fungi, but did not affect the degree of plant infestation (Sucharzewska, Dynowska, Ejdys, et al., 2012).

Continuous research on hyperparasitism, especially an assessment of the prevalence of *A. quisqualis* and its impact on host growth in the urban environment, showed that this hyperparasite finds very favorable conditions for growth on the mycelium of Erysiphales fungi. Data obtained from the available literature showed a low share of the species in the natural environment, such as natural reserves or national parks, or even no records at all (Majewski, 1971). Based on the documented observations, *A. quisqualis* was classified as an urbanophil. The high prevalence of mycoparasites of the genus *Ampelomyces*, with a simultaneous high prevalence of Erysiphales fungi, confirmed earlier conclusions that the presence of *A. quisqualis* in natural conditions neither considerably limits the host plant infestation by Erysiphales nor disrupts their life cycle (Sucharzewska et al., 2011). *Ampelomyces quisqualis* was found most frequently in young fruit bodies of Erysiphales fungi, but with *Erysiphe flexuosa* on *Aesculus hippocastanum* and *E. vanbruntiana* var. *sambuci-racemosae* on *Sambucus racemosa*; conidiospores of the hyperparasite were also found inside mature fruit bodies. This was the first description of this phenomenon. International literature only provided information on *A. quisqualis* settling on young fruiting bodies and transforming them into their own reproductive structures. The presence of conidiospores inside fruit bodies with properly developed rhizines suggested an improvement in the adaptive strategy (Sucharzewska, Dynowska, Kubiak, et al., 2012). Colonization of mature chasmothecia, including the completion of peridium formation with properly developed rhizines, creates proper conditions for hyperparasites for better wintering and propagation in the environment. This may result in an increase in the presence of *A. quisqualis* in the urban environment, and in consequence, lead to changes in the Erysiphales population. An analysis of the relationship of Erysiphales with other fungi (*Alternaria*, *Cladosporium*, *Stemphylium*), noted in earlier studies, was also conducted. The use of electron microscope techniques (project No. 2017/01/X/NZ8/00798) confirmed a parasitic relation between Erysiphales spp. and the fungi present on their surface. Hyperparasites destroyed the colonized development stages of Erysiphales, and external and internal hyperparasitisms were observed. In the case of the former, numerous structures of parasitic fungi were found on the surface of the pathogens at the development and accompanying cell lysis stages. In the case of the latter, parasites were present inside the pathogens at the sexual and asexual stages, e.g., the presence of hyphae of *Stemphylium sarciniforme* in the rhizines of fruit bodies of *E. palczewskii* (Sucharzewska, 2019).

The development of parasitic fungi can also be affected by organisms that do not directly interact with them. Several species compete for nutrients and habitat. This is the case with *E. flexuosa*, which infests the horse-chestnut tree and a butterfly called horse-chestnut leaf miner (*Cameraria ohridella*), which feeds on this plant species. Both species colonize the plant leaves and compete for the most beneficial living conditions. The observation of the development cycle of *E. flexuosa* on horse-chestnut tree leaves in an urban environment, taking into account the presence of *C. ohridella*, led to several conclusions. A high level of presence of the species under study on two horse-chestnut species (*A. hippocastanum* and *A. × carnea*), with a distinct preference of *E. flexuosa* for *A. × carnea*, and the butterfly for *A. hippocastanum*, was observed. *Erysiphe flexuosa* may have completed its life cycle, but the co-occurrence with *C. ohridella* affected the disease index and the number of fruit bodies formed by the fungus. Analyses showed that the differences in preference of hosts ensure that *E. flexuosa* has proper conditions for growth and formation of a large number of fruit bodies with spores, which, as a result of the sexual process, enable the parasite to adapt to changing environmental conditions, which are particularly present in urbicenos. Therefore, it was stressed that research focusing mainly on controlling the horse-chestnut leaf miner should also take into account the presence of *E. flexuosa*, which, because of its invasiveness, poses a serious threat to horse-chestnut trees (Sucharzewska et al., 2018).

Long-term monitoring conducted at the Department of Mycology revealed the presence of numerous parasitic fungi and fungi-like organisms on urban plants. Altogether, over 300 species were identified (individual groups: Pucciniales, 45%; Erysiphales, 22%; and others, 33%). Among the identified species, there are several regarded as interesting from the perspective of Poland: *Entomosporium mespili*, *Erysiphe deutziae*, *Kuehneola uredinis*, *Monilia linhartiana*, *Phyllosticta juglandis*, and *Seimatosporium lichenicola*. Some new hosts were found, e.g., *Juglans nigra* for *Ascochyta juglandis*, *Quercus rubra* for *Discosia artocreas*, *Ginkgo biloba* for *Epicoccum purpurascens*, *Juniperus communis* for *Pestalotia guepinii*, and *Crataegus monogyna* for *Seimatosporium lichenicola*. *Erysiphe trifolii* was driven out from *C. arborescens* by *E. palczewskii* (Sucharzewska et al., 2011).

Despite reports on the species diversity and changes of parasitic mycobiota on plants in Polish cities, these issues are still poorly explored. Owing to its dynamic character, urbicenos is a perfect research ground on which numerous interactions and changes in the “parasite–host” and “host–parasite–hyperparasite” systems result in the perfection of the life strategies of parasites, with consequent preservation of the species.

Although studies of fungi ecophysiology in the urban environment were initiated just after the research teams at the Department were formed, their dynamic growth started with the doctoral thesis entitled *Strategie życiowe wybranych grzybów z rzędu Erysiphales w warunkach zróżnicowanej antropopresji* [Life strategies of selected Erysiphales fungi under variable anthropopressure] (Sucharzewska, 2007). The findings to date have been published in 35 papers, most of which are original scientific articles. Recently, field research aimed at analyzing parasitic fungi of the phyllosphere of aquatic plants in the littoral of selected lakes in Olsztyn was completed. It took into account the functioning of the fungi with respect to urban pollution and biomonitoring.

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