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DYNAMICS OF FUNGI AGAINST THE BACKGROUND OF HOST PLANT PHENOLOGY. PART I. LIST OF MICROFUNGI INFECTING *STELLARIA HOLOSTEA*[*]

WIESŁAW MUŁENKO & MONIKA KOZŁOWSKA

Abstract. The paper analyzed the occurrence of microfungi on *Stellaria holostea* (Caryophyllaceae), a typical component of the field layer in deciduous and mixed forests in Central Europe, especially oak-linden-hornbeam forests (*Tilio-Carpinetum*). Investigations were conducted in the Białowieża Primeval Forest at a permanent research plot in Białowieża National Park with well-documented ecology. A total of 22 species of microfungi were collected on *S. holostea* over a period of four years. Their occurrence was examined throughout the vegetative season and in relation to the plant's phenological development. Fungi included species new to Poland, rare species, and species for which *S. holostea* is a new host.

Key words: *Stellaria holostea*, microfungi, parasites, saprobes, ecology, oak-linden-hornbeam forest, *Tilio-Carpinetum*, Białowieża National Park, Poland

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INTRODUCTION

Fungal species richness and diversity have always interested mycologists. The first documented data were published in studies by Fries, who estimated the number of fungi to be the same as that of plants. A survey by Hawksworth (1991), who traced the total number of fungi based on the number of plants occurring in specific areas, triggered a particularly heated debate. Numerous works elaborating the issue worldwide have followed since (e.g., Cannon & Hawksworth 1995; Hyde 1996; Hawksworth & Rossman 1997; Dulymamode *et al*. 2001; Hawksworth 2001, 2004; Kohlmeyer & Volkmann-Kohlmeyer 2001; Wong & Hyde 2001; Yanna *et al.* 2001).

These studies provide interesting information on the total number of fungi found in specific areas and also on individual species of plants. They also note that species of practical interest to people, cultivated species in particular, have been examined considerably more than those growing in the wild. Much less is known about the latter group. Investigations in natural conditions are very infrequent and concentrate on only a few plant species. Although many of them are very important components of the environment, the composition of fungi colonizing them is not well recognized. An impressive number of fungal species have been recorded so far on individual plant species, both cultivated and growing in the wild. For instance, 135 species of fungi are known to occur on *Oryza sativa*, 92 on *Urtica dioica*, 55 on *Lantana camara*, 117 on *Juncus roemerianus* (Hawksworth 2001), 99 on *Dryas octopetala* (Chlebicki 2002) and 170 on reeds (*Phragmites* spp.) (Wong & Hyde 2001). Considerably more are reported for deciduous species, which can be occupied by hundreds or even thousands of fungal species: for example, 893 species on *Pinus sylvestris*, 590 on *Quercus suber*, and 282 on *Eucalyptus globosus* (Hawksworth 2001).

Research on widespread plants that form plant associations of natural types should be given special priority. Such studies are still too few, especially

[*] This paper is dedicated to Professor Tomasz Majewski on the occasion of his 70th birthday.

in Central Europe. Researchers are often attracted by tropical and subtropical regions which have not been explored intensively.

Much information on the number of fungi occurring on plants can be found in taxonomic monographs and on checklists of fungi dealing with specific countries, regions or continents, often published together with keys to their determination (e.g., Brandenburger 1985; Farr *et al*. 1989; Mułenko *et al*. 2008). Ecological-floristic examinations of natural plant communities are also important contributions (e.g., Mułenko 1988, 1995; Ruszkiewicz-Michalska 2006).

The present studies are detailed ecological investigations focusing on the dynamics of the occurrence of microfungi on plants that are components of oak-linden-hornbeam forest (*Tilio-Carpinetum*). A total of 30 species of plants forming the association were examined. Oak-linden-hornbeam forest is the most important association in the Białowieża Primeval Forest and potentially also in lowland areas in Poland and Central Europe. An important species is *Stellaria holostea* (Caryophyllaceae), which is a characteristic element of deciduous and mixed forest occurring across almost the entire European continent. The biology of its development, wide occurrence, persistence and other properties of its structure make it a model species for analyses of this type. Investigations were conducted in Białowieża National Park (Białowieża Primeval Forest) the most natural forest area in Central Europe.

This study opens a series analyzing the occurrence of microscopic fungi in relation to the phenological development of plants. Further studies will investigate other plant species forming *Tilio-Carpinetum*. Selected results of research into *Stellaria holostea* have already been published, but only as preliminary data (Mułenko 1998; Kozłowska & Mułenko 2003, 2004; Mułenko & Kozłowska 2003). Some fungi have lately been revised again and the database has been updated, all of which affects how the results are interpreted and presented.

Here we give a list of species and analyze the distribution of fungi in the study area. Successive analyses will be discussed in further papers. Microfungi have not been researched in terms of the internal structure of one community. Attempts of this type dealt only with macrofungi (Nespiak 1968).

Subject of study

Host plant. *Stellaria holostea* (Caryophyllaceae) is a geophyte producing underground rhizomes. It is classified as a herbaceous chamaephyte in Ruankiaer's system of life forms. *S. holostea* is one of the most important differential species of deciduous forests (oak-linden-hornbeam), characteristic of the entire *Carpinion* alliance. It reaches maximum constancy and abundance in the association *Stellario holosteae-Carpinetum betuli* (Matuszkiewicz 2001, 2007). The range of dense occurrence of *S. holostea* comprises nearly all of Europe, from Great Britain and France in the west to the Urals in the east. Longitudinally it stretches from the northern borders of Portugal and Spain, across Italy and the Balkans in southern Europe, up to the Baltic Sea in the north. *S. holostea* is also noted at individual localities in the southern part of the Scandinavian Peninsula (Hultèn & Fries 1986).

In Poland, *Stellaria holostea* occurs at numerous localities in many regions, in almost the entire lowland up to the upper forest limit in the mountains. Its typical habitat conditions include fresh soils, moderately acidic and neutral. It prefers moderately warm climatic conditions, semi-shady localities, and it occurs commonly in forests and shrubs (Gibbons & Brough 1995; Zając & Zając 2001; Zarzycki *et al*. 2002).

Stellaria holostea is considered the most typical plant of *Tilio-Carpinetum*. It was selected for this study due to its widespread occurrence and the biology of its development. It is recorded in all 100 squares of the study area and grows throughout the vegetative season. Undecomposed plant remains from the previous year, colonized by fungi, are also often seen in early spring.

Fungi. Microscopic fungi and fungi-like organisms (pseudofungi) recorded on aboveground organs of *Stellaria holostea* were investigated. Fungi occurring on roots were not collected, and so-called endophytes were not isolated. The fungi studied belong to several taxonomic groups. True

fungi are represented mostly by rust fungi (Pucciniales, Basidiomycota), ascomycetous fungi (Ascomycota, 2 orders) and anamorphic fungi (Hyphomycetes and Coelomycetes). Fungi-like organisms belong to Oomycota (order Peronosporales). For practical reasons, the organisms listed here will be referred to by the common name 'fungi'.

Study area

Investigations were conducted in the Białowieża Primeval Forest in the central part of Białowieża National Park. It is one of the most impressive forest areas of natural origin in the lowland part of Europe, the object of model studies on the functioning of natural forest ecosystems (Faliński 1968a; Faliński & Hereźniak 1977). It is the first transborder world heritage site in Europe, with naturally developed plant cover, rich fauna and diversified terrain (Faliński 1968b; Faliński & Okołów 1968; Denisiuk & Witkowski 1990; Mirek *et al*. 2005).

Detailed investigations were conducted in the *Tilio-Carpinetum* forest association (oak-linden-hornbeam). The association plays a special role in the ecosystem. It covers *ca* 40% of the Białowieża Primeval Forest and is the most important community in the area. It should also potentially dominate all of lowland Poland. It is a multilayer mixed forest with a multi-species tree stand. The most important species are common hornbeam (*Carpinus betulus*), small-leaved linden (*Tilia cordata*) and pedunculate oak (*Quercus robur*). The shrub layer is diversified and the field layer is rich in species, with a high contribution of geophytes.

A permanent research plot was established in the association in section 342 of Białowieża National Park (plot no. 40 of the Białowieża Geobotanical Station, University of Warsaw). The plot covers 1 ha comprising oak-linden-hornbeam forest (*Tilio-Carpinetum*) divided into one hundred squares (100 m^2 each), with excellent ecological documentation (Fig. 1). It is representative of oak-linden-hornbeam forests of the entire area, as it contains the two most characteristic plant subcommunities: *T-C typicum* and *T-C stachyetosum*. It is also probably the most homogeneous part of the oak-linden-hornbeam forest in 20 km^2, that is, almost 50% of the area covered by the association in Białowieża National Park (Faliński 1966).

The plot is occupied mostly by two subcommunities: *T-C typicum*, occurring in the northern, western and southern parts (51 squares), and *T-C stachyetosum* in the central and eastern parts (27 squares). Very small areas of another subcommunity, *T-C calamagrostietosum*, were recorded in two squares (squares 1 and 11) (Faliński 1966). Only *T-C typicum* and *T-C stachyetosum* were included in the analysis, due to their size. Squares forming the transition zone between the two were identified (ecotone, 22 squares) (Fig. 1), and additional analyses of the distribution and ecological preferences of fungal species were conducted.

Aim of the study

The main purpose of this research was to identify the rhythm of occurrence of microfungi in relation to the phenological development of plants forming

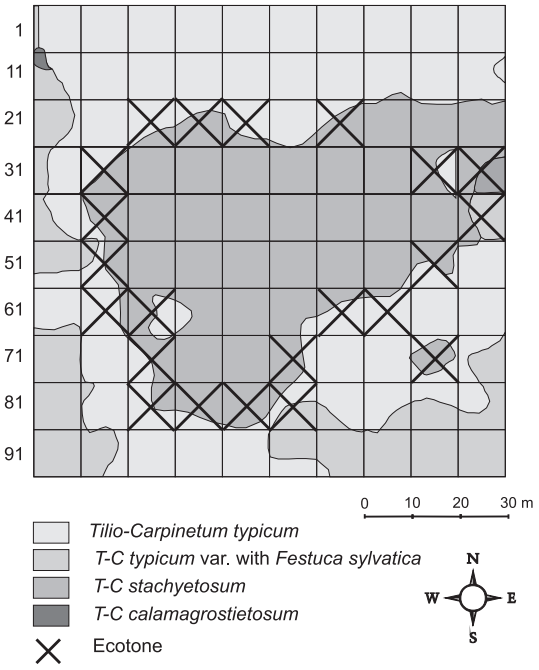

Fig. 1. Diagram of the study area (after Faliński 1966, modified).

the oak-linden-hornbeam association (*Tilio-Carpinetum*). As abundant material was collected, the results will be published successively as narrow-focus studies on individual plant species.

The aim of this study was to analyze the occurrence of fungi recorded on *Stellaria holostea*. Issues discussed here include (*i*) the distribution of fungi in relation to the distribution of plants, (*ii*) the development of fungi during the vegetative season, (*iii*) the frequency of fungal occurrence, and (*iv*) preferences of fungi for selected *Tilio-Carpinetum* subcommunities, the ecotone and land relief forms. Notes on the distribution of fungi in Poland and worldwide precede further analyses.

Study period and methods

Field investigations

Field investigations were conducted between 1992 and 1995. Preliminary studies were done in the first year, followed by two years of investigations and one year of supplementary studies. Infected plant specimens were collected in each square, twice a month on average, during the entire vegetative season from April until November. Fungi were collected on aboveground plant organs, mostly on leaves and flowers, less frequently on stems. At least five specimens of infected plants were collected in each square for identification purposes.

An inventory of the occurrence of *Stellaria holostea* in each of the squares was made in the first year. It was partly supplemented in successive years and slight corrections were made. The frequency of *S. holostea* occurrence (defined as the degree of cover of the area) is given on a five-degree scale with the following classes: 1 – very rare (< 10%), 2 – rare (10–20%), 3 – frequent (21–40%), 4 – very frequent (41–60%), 5 – en masse (> 60%).

The frequency of fungi, defined as the percentage of infected individuals of *S. holostea*, was also estimated in each square. A comparative five-degree frequency scale was used: 1 – very rare (< 1% infected plants), 2 – rare (1–10%), 3 – frequent (11–30%), 4 – very frequent (31–60%), 5 – en masse (> 60%). Both frequency scales were discussed in detail elsewhere (Mułenko 1997).

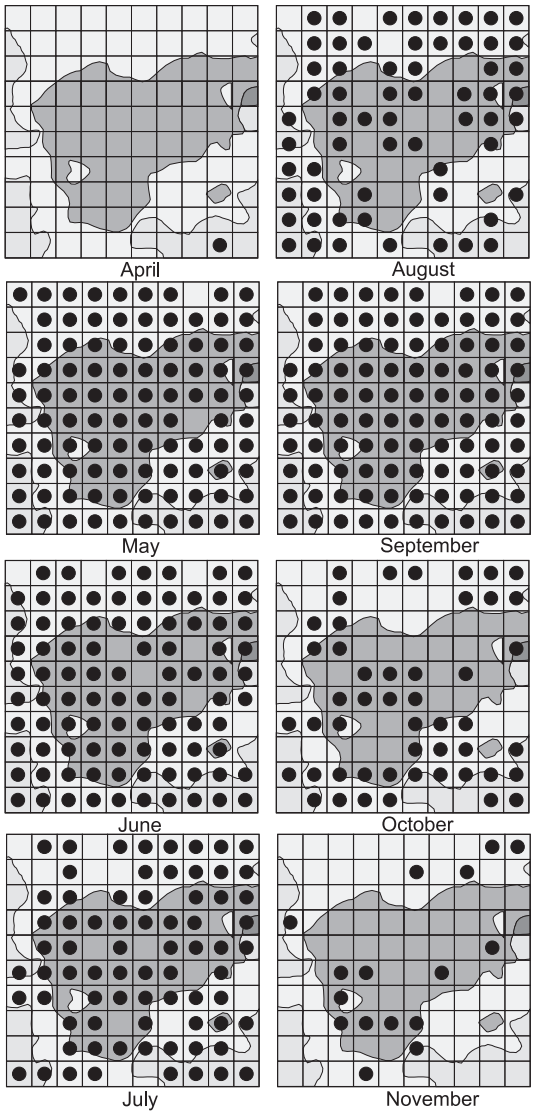

Fig. 2. Distribution of *Phacelium episphaerium*.

The statistical method used to calculate (1) the distribution at the plot and (2) the number of records needs some clarification:

(1) The number of squares at the plot is 100. Thus, the maximum number of localities of a fungus at the plot cannot exceed 100 (100%), regardless of the period of its occurrence or the number of records during a season or in the study

period. The actual number of localities was used to calculate preferences for individual subcommunities and the ecotone. The number of localities (squares) occupied by them was taken as 100% in this case.

(2) Core investigations were conducted for two years, during which each square was studied twice per month. Given the number of squares (100) and the collection period (8 months), the maximum number of records in a year should theoretically be 1600, assuming that a plant and a fungus occur during the entire season and at all the localities. These numbers were much lower in practice, as not all the species of plants and fungi were present throughout the vegetative season and the collection period. Furthermore, data collected in each square were amassed to one record per month, accepting the greatest frequency, which is included in the database, retaining all the herbarium collections at the same time. The maximum number of records presented in the study is then the sum of the localities of a fungus in successive months. The manner of the occurrence of *Phacellium episphaerium* (Fig. 2), a species common in the study area, developing throughout the season but at different intensity in each month, is used as an example. The total sum of records of *P. episphaerium* is 483, which is 100% when the proportional contribution to individual frequency classes and the preferences for terrain microforms are calculated (Fig. 4B).

The occurrence of fungi in a variety of terrain types formed by different biotic and abiotic factors was also noted and those fungi were collected during investigations. Terrain microforms included wild boar rooting sites, hummocks and hollows after wind-fallen trees. The classification of terrain microforms is given below. The same reference numbers are used in the figures.

Terrain microforms (see also Fig. 4 A–X):

Primary abiotic microforms [1–3]
- 1 – flat forest floor
- 2 – (hummocks) small hills
- 3 – (hollows) small depressions

Secondary biotic microforms [4–6]
- 4 – hummocks after fallen trees
- 5 – hollows after fallen trees
- 6 – wild boar rooting sites
- 7 – plants growing on tree trunks and logs.

A series of maps with the distribution and density of the host plant is given in the final part of the study (Fig. 3), and the occurrence of fungi is contextualized by maps. All of the species discussed in the study are mapped, regardless of the number of localities; the highest (for a specific locality) frequency class is indicated (Fig. 4A–X). Maps are arranged in descending order by number of records. The following information is given for each map:

– the occurrence of the fungus in successive months of the vegetative season from April until November (number of localities where the fungus was collected);

– the preferences of the fungus for individual subcommunities and the ecotone (number of squares for each was taken as 100%);

– the number of records in individual frequency classes (percentage contribution is also given) and the total sum of records of the fungus;

– the occurrence of the fungus within terrain microforms and on plants growing on tree trunks and logs (total number of records of a specific fungal species was taken as 100%).

LABORATORY INVESTIGATIONS

Fresh material or dried herbarium specimens were used for morphological observations. A small piece of living tissue containing fungal structures was mounted in water for microscopic examination. The dried specimens were rehydrated in 3% KOH solution and then observed. Measurements of conidiophores and conidia for each sample were made from 40–50 examples at 400× and 1000× with an eye-piece micrometer under a light microscope (Olympus BX41), and images were made with an ARTRAY camera. All dried specimens are housed in LBL, Maria Curie-Skłodowska University, Lublin, Poland.

The fungi were determined using the following studies: Brandenburger (1985), Braun (1995, 1998), Constantinescu (1991), Crous and Braun (2003), Ellis (1971, 1976), Ellis and Ellis (1987),

Kochman and Majewski (1970, 1973), Majewski (1977, 1979), Mel'nik (2000), Morochovski *et al*. (1971), Nag Raj (1993), Sałata (2002), Sutton (1980), Teterevnikova-Babayan (1987), Vanev *et al*. (1997) and Wołczańska (2005).

SYSTEMATIC LIST OF FUNGI

The systematic arrangement of fungi and fungi-like organisms follows the most recent edition of *Dictionary of the Fungi* (Kirk *et al*. 2008), with the exception of anamorphic fungi ('Deuteromycota'), for which the traditional approach was used. Fungi are arranged alphabetically within orders.

Kingdom: Chromista
- Phylum: Oomycota
  - Class: Oomycetes
    - Order: Peronosporales

Kingdom: Fungi
- Phylum: Ascomycota
  - Class: Ascomycetes
    - Order: Mycosphaerellales
    - Order: Pleosporales
- Phylum: Basidiomycota
  - Class: Basidiomycetes
    - Order: Uredinales

Anamorfic fungi ('Deuteromycota')
- Class: Hyphomycetes
  - Order: Moniliales
- Class: Coelomycetes
  - Order: Melanconiales
  - Order: Sphaeropsidales

RESULTS

Peronosporales

Peronospora parva Gäumann

Peronospora parva is quite a rare fungal species. Only 11 records on its occurrence in the world, Europe and Asia are available in the fungal databases. It was recorded on five species of the genus *Stellaria* and on *Fagopyrum esculentum*, but was collected at only few localities (Farr & Rossman 2010).

In Poland, *P. parva* is known from five localities. Outside the Białowieża Primeval Forest (Kochman & Majewski 1970; Majewski 1971; Mułenko 1996) it was also collected in Wołowo and Skierniewice (Kochman & Majewski 1970), in the Jezioro Długie Lake Reserve in the Pojezierze Łęczyńsko-Włodawskie lake district (Mułenko 1988) and in Roztocze National Park (Chmiel *et al*. 1994). The results of investigations in Poland so far indicate that the fungus occurs more frequently but has not been examined in detail. For instance, it was collected at 24 localities in one forest section (140 ha) during investigations in the Białowieża Primeval Forest (Crypto Program, Faliński & Mułenko 1997), and was recorded at 72 localities in this study.

Peronospora parva is a biotrophic parasite which infects only living leaves of the host plant. Development of the fungus occurred nearly throughout the vegetative season. The first infection symptoms appeared as early as May as young plants developed, and the number of records was highest in May (49). It persisted at the same localities for most of the season and its distribution fluctuated only slightly. The number of localities dropped suddenly only in autumn (October, November). It occurred frequently at the plot (72 localities) but mostly in the lowest frequency class (1 – very rare, 98 records, 50%). The analysis shows that *P. parva* occurred in the ecotone (18 localities in 22 squares, 82% contribution) more frequently than in the subcommunities (69% in *T-C typicum*, 70% in *T-C stachyetosum*) (Fig. 4F).

Mycosphaerellales

Mycosphaerella isariophora (Desmazières) Johanson

World data on *Mycosphaerella isariophora* are quite sparse. The fungal databases give the distribution of only two host species of the genus *Stellaria* [*S. holostea* L., *S. media* (L.) Vill] reported from three European countries and on *Orthilia secunda* (L.) House (= *Pyrola secunda* L.) from the U.S.A. It seems that *M. isariophora* is considerably more frequent and that the information on its occurrence has not been updated recently. For instance, it is reported from Iceland on six species of plants belonging to the family Caryophyllaceae (Hallgrímsson & Eyjólfs-

dóttir 2004), and Desprez-Loustau (2009) places it on the list of invasive species alien to Europe. In Poland it was recorded on *Stellaria holostea* L. at a few localities in Silesia (Schroeter 1908), Kielce (Moesz 1926) and the Białowieża Primeval Forest (Faliński & Mułenko 1997).

Mycosphaerella isariophora is the most numerous species of fungi collected in the study area. It was also noted throughout the year. Infection symptoms occurred on young plant leaves in May, although initially at few localities (19). The number of records suddenly increased in June and remained very high until the end of the season, reaching maximum in September and October. At the end of the season the fungus occurred only on dying or dead plant fragments that persisted through the winter to the following year. It was also collected in April on plant remains from the previous year.

Mycosphaerella isariophora had the highest number of records (525) in the study period. Its frequency in individual squares was also high. It was recorded mostly in the third frequency class (frequent). The fungus was not recorded from only two localities in the northwestern part of *T-C typicum* (Fig. 4A). Its contribution to each of the subcommunities and the ecotone was similarly high: 96% in *T-C typicum*, 100% in *T-C stachyetosum*, 100% in ecotone (Fig. 4A).

It is interesting that the fungus occurred very often near *Phacelium episphaerium* or even at the same sites, causing combined infections. *Phacellium* is not the anamorphic stage of *M. isariophora*, however.

Pleosporales

Didymella holosteae Sydow

The fungus is associated solely with *Stellaria holostea* and is very rare. Its only locality in Poland is in Białowieża National Park (Mułenko 1998), and it is also known from a few localities worldwide. Its occurrence has been reported from Germany (Herbarium Hamburgense, HBG, after *Index Fungorum*), Great Britain (Slapton Nature Reserve, Hawksworth & Sivanesan 1975) and Russia (Farr & Rossman 2010).

The occurrence of *Didymella holosteae* was confirmed at 49 localities in the present study, and it was recorded 71 times during the collections. It is therefore not rare but requires more in-depth investigations. It was collected throughout the vegetative season, usually on dying or already dead leaves of plants. The number of records was initially small but gradually increased in the following months (from 1 to 9), reaching maximum in September (28). Its distribution decreased towards the end of the collections. There were 13 records in October and only eight in November. The fungus was recorded only in the lowest frequency class (very rare).

The distribution range of *D. holosteae* comprises nearly 50% of the study area. The localities are spread uniformly, with fewer only in the northern and northwestern parts of the plot. It was recorded more often in *T-C stachyetosum* and in the transition zone, and considerably less frequently in *T-C typicum* (Fig. 4H).

Leptosphaeria stellariae Rostrup

It is a very rare species, recorded in Poland only in Białowieża National Park (Mułenko 1996; Faliński & Mułenko 1997). It is reported worldwide on four species of the genus *Arenaria* and six species of the genus *Stellaria* in seven countries: Poland, France, Denmark, Sweden, Greenland, Canada and the U.S. (Alaska) (Farr & Rossman 2010).

The period of its occurrence in the plot was relatively long but its distribution was very small. It was recorded only at single localities over the few months after its first occurrence (V–VIII). Its distribution was slightly higher only in September (five localities). The fungus was recorded in the lowest frequency class. *Leptosphaeria stellariae* was recorded at nine localities distributed mostly in the southern and central parts of the plot. It was recorded slightly more frequently only in the transition zone (Fig. 4S).

Leptosphaeria sp.

The fungus was recorded on dying discolored plant leaves. Perithecia (280) 320 (360) µm in

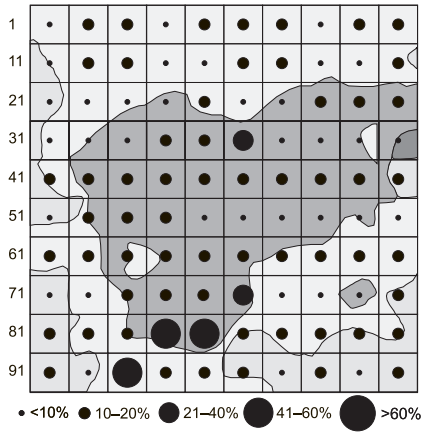

Fig. 3. Distribution and density of *Stellaria holostea* L.

diameter. Asci numerous, cylindric-clavate, 60–80 × 6–8 µm, 8-spored. Ascospores 25–32 × 3 µm, mostly 30 × 3 µm, 5–8-septate. Spores constricted at one end. Some spores with one cell delicately enlarged. Some spores with 3 cells longer than others at the narrower end. A fungus with this description was not previously reported among the species of the genus *Leptosphaeria* and must be studied further to provide a full description.

The fungus was recorded over a period of six months, from June to November. It was collected at a total of 19 localities distributed in the southern and central parts of the plot, but only in the lowest frequency class. The number of records was also low (total 23), and ranged from one to

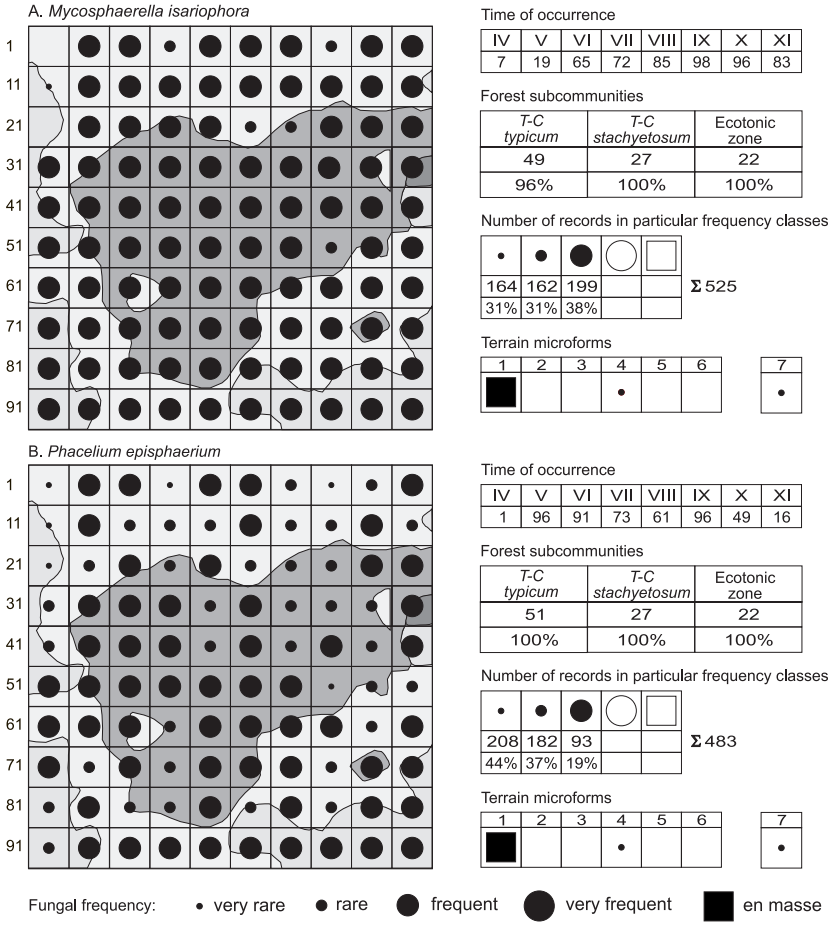

Fig. 4A & B. Occurrence of microfungi on *Stellaria holostea* L. (very rare – <1%, rare – 1–10%, frequent – 11–30%, very frequent – 31–60%, en masse >60%).

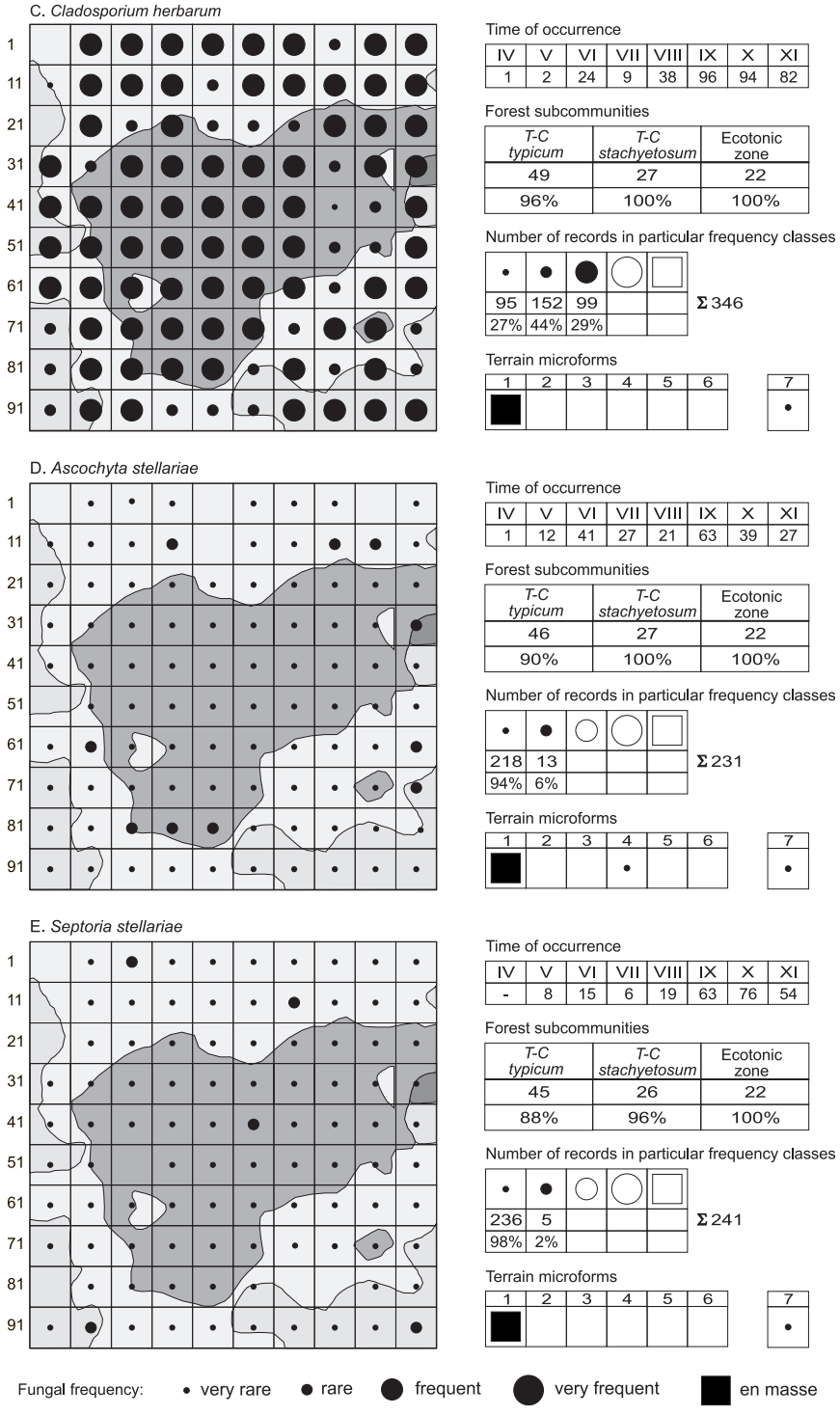

Fig. 4C–E. Occurrence of microfungi on *Stellaria holostea* L. (*continued*).

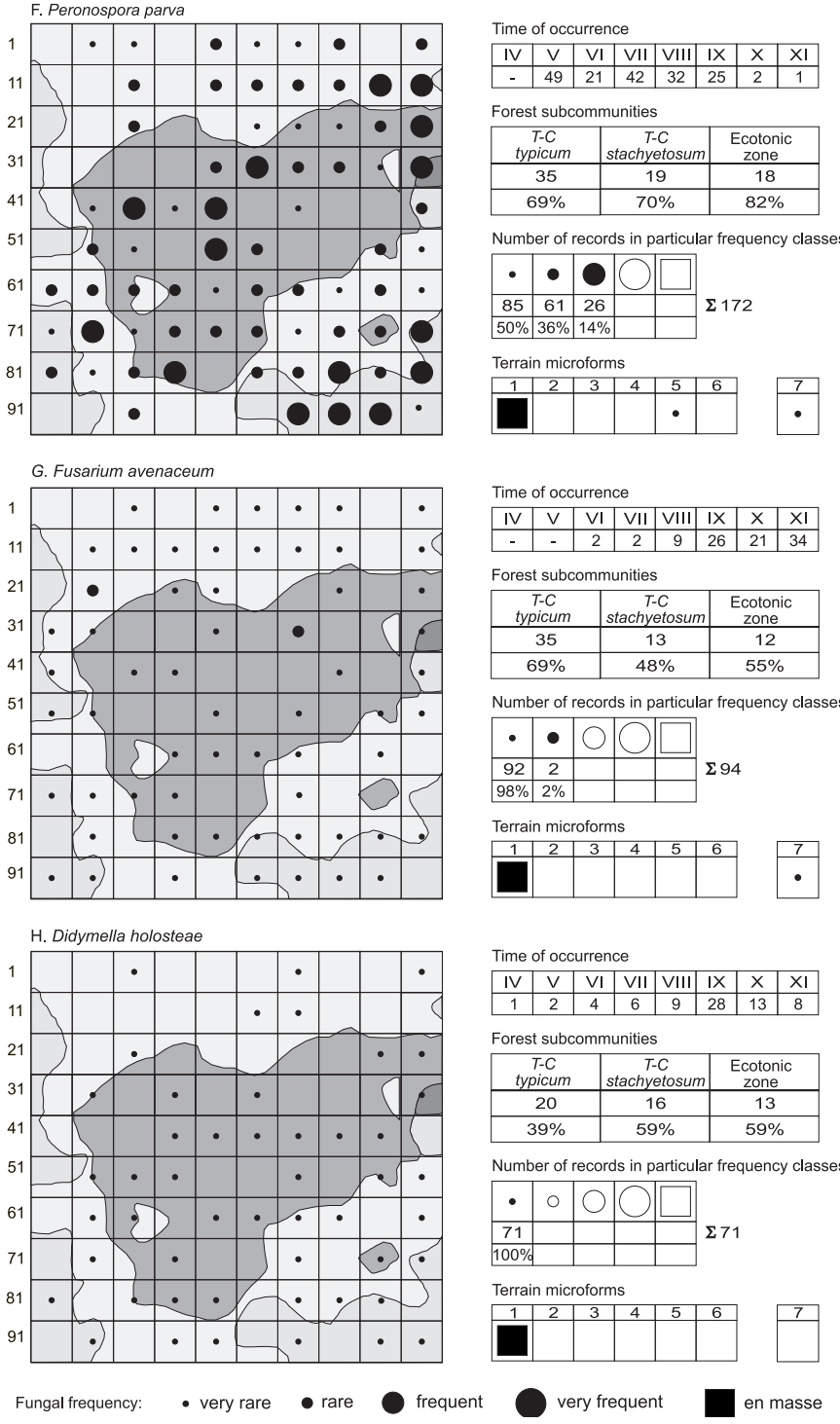

Fig. 4F–H. Occurrence of microfungi on *Stellaria holostea* L. (*continued*).

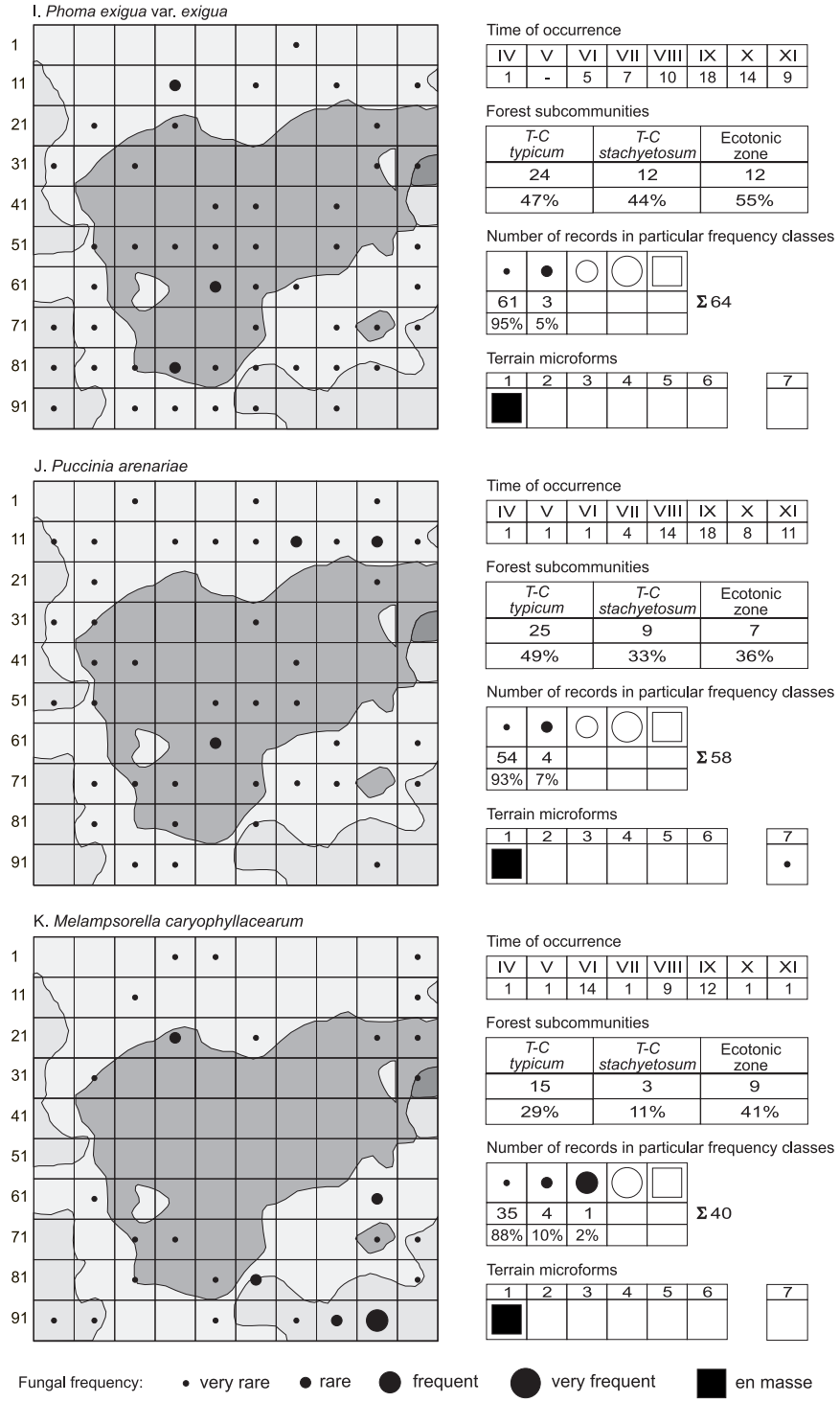

Fig. 4I–K. Occurrence of microfungi on *Stellaria holostea* L. (*continued*).

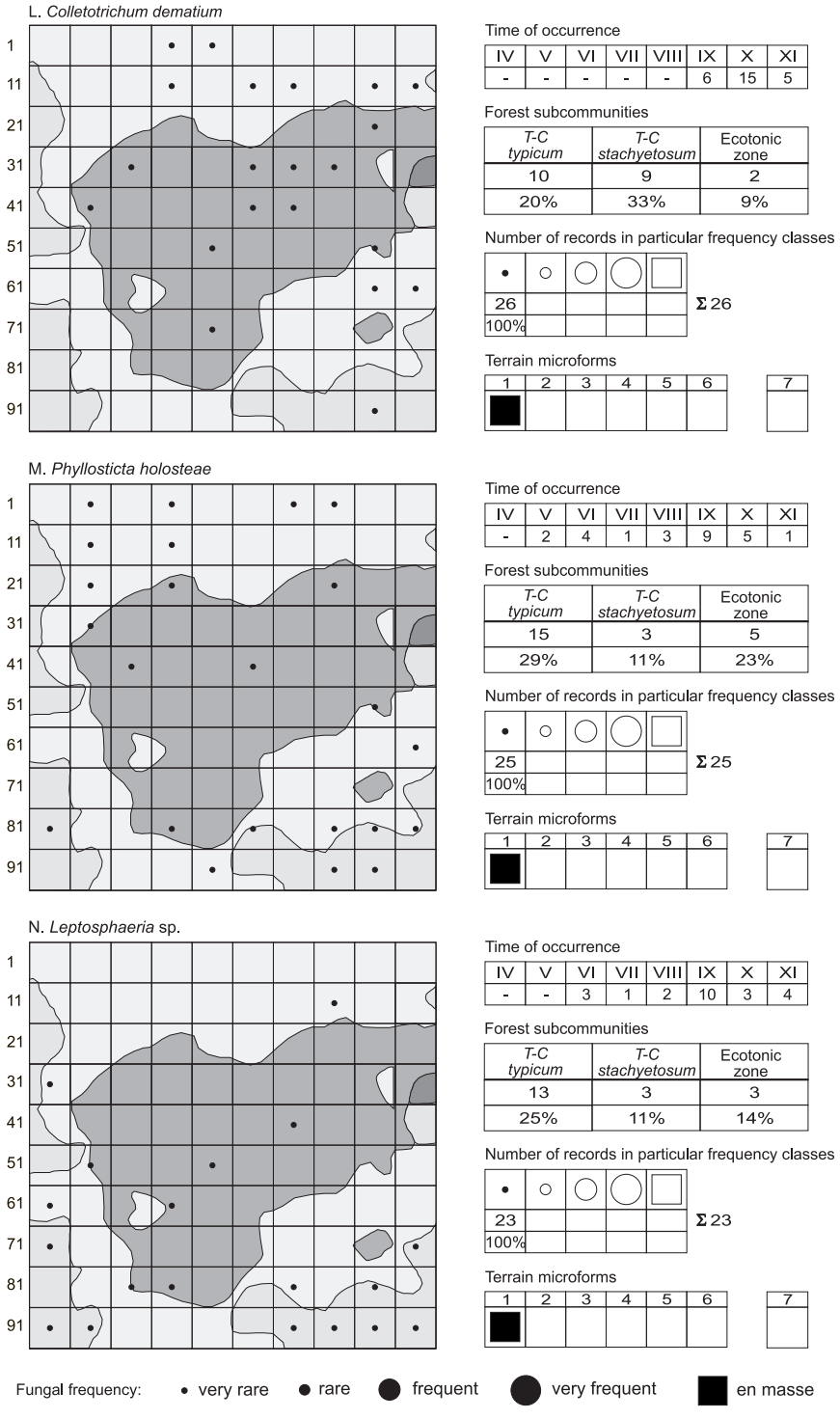

Fig. 4L–N. Occurrence of microfungi on *Stellaria holostea* L. (*continued*).

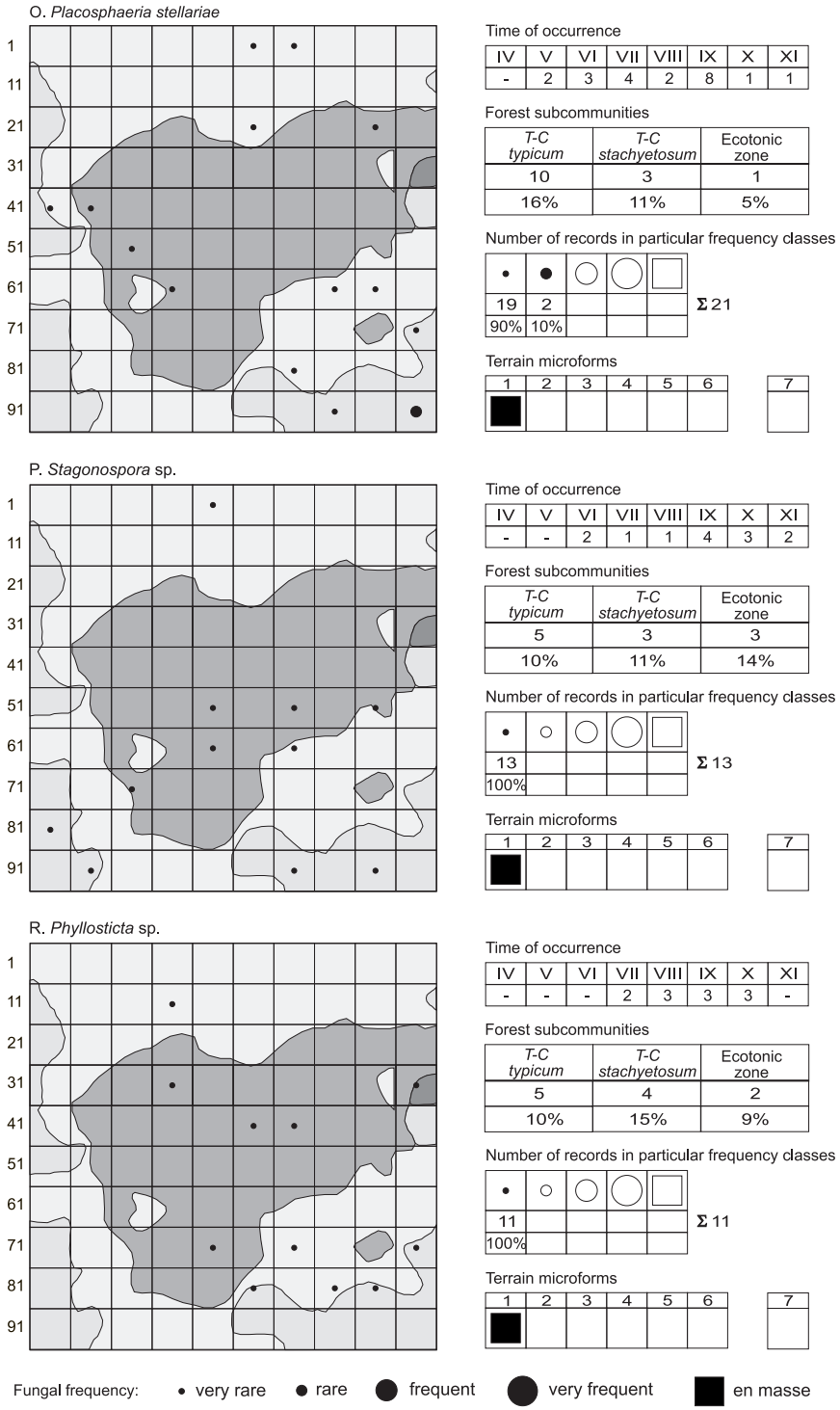

Fig. 4O–R. Occurrence of microfungi on *Stellaria holostea* L. (*continued*).

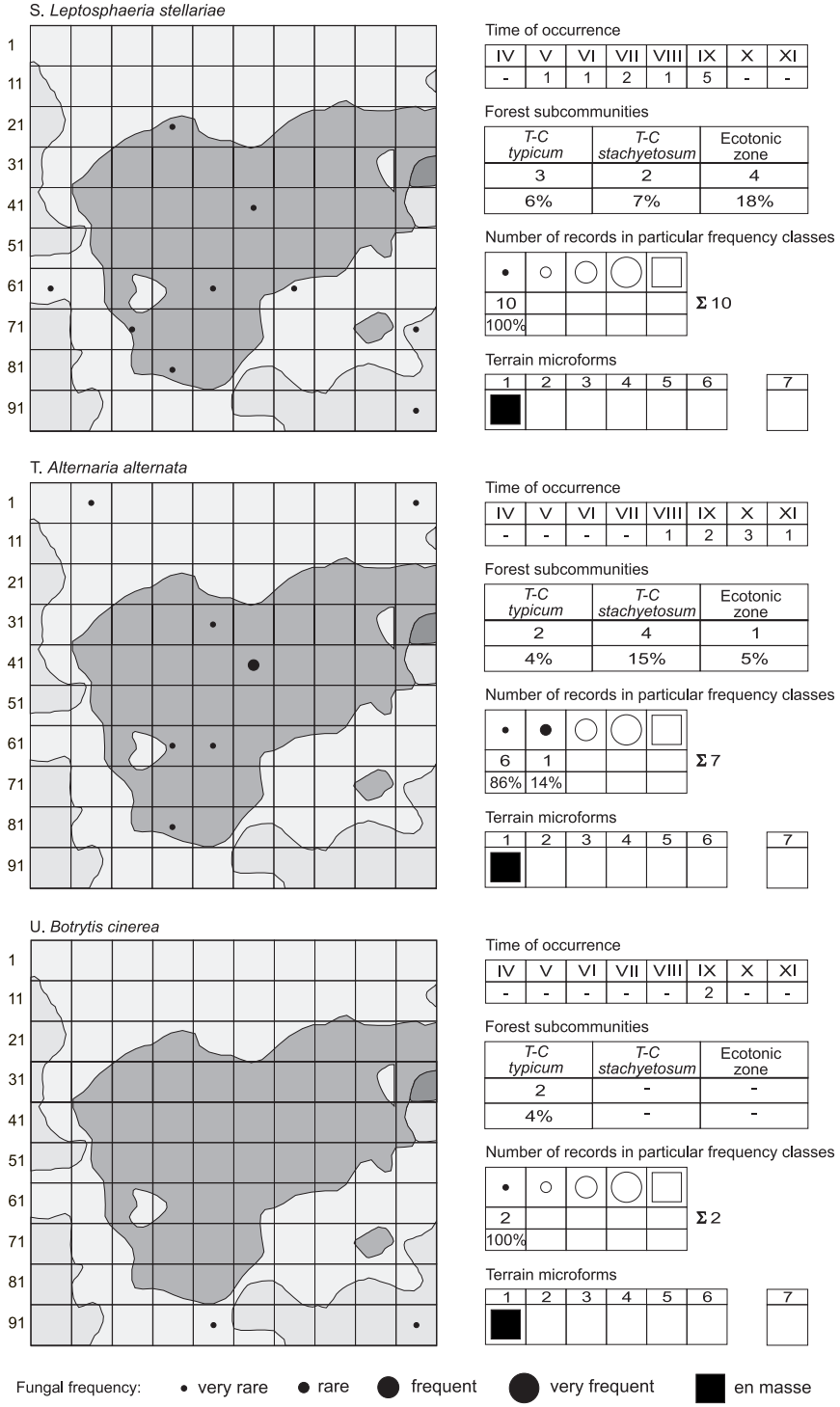

Fig. 4S–U. Occurrence of microfungi on *Stellaria holostea* L. (*continued*).

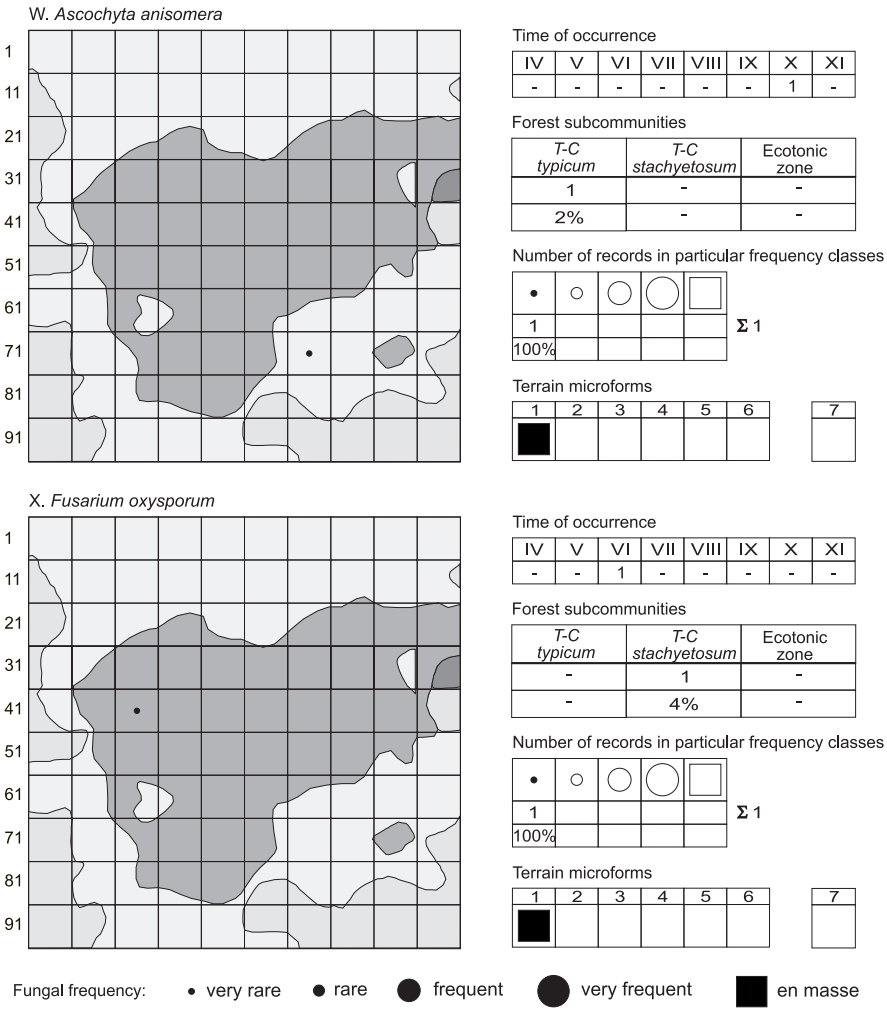

Fig. 4W & X. Occurrence of microfungi on *Stellaria holostea* L. (*continued*).

four during the vegetative season. It was recorded at ten localities only in September. The majority of localities (13) were in *T-C typicum* (25% of the area occupied by the subcommunity). It was recorded very rarely in *T-C stachyetosum* and in the ecotone (Fig. 4N).

Uredinales

Melampsorella caryophyllacearum Schroeter

It is a two-host species. The aecial stage occurs on firs (*Abies* spp., Pinaceae), producing so-called witches' brooms. The uredinial and telial stage infects representatives of the family Caryophyllaceae, sometimes causing systemic infections of plants. It has been recorded on five species of the genus *Abies* and many species of the genera *Cerastium*, *Myosoton* and *Stellaria* worldwide (Ellis & Ellis 1987). Over 600 records worldwide are reported in the fungal databases (Farr & Rossman 2010). In Poland it was recorded on 11 plant species and at many localities in a variety of regions (Majewski 1977; Majewski & Ruszkiewicz-Michalska 2008).

The fungus was observed in the study area throughout the vegetative season from April until

November but it was not frequent. It was recorded at a total of 27 localities, and the greatest number of occurrences was noted in June (14), followed by August (9) and September (12). It was recorded at single localities in other months and mostly in the lowest frequency class (88% of records as very rare).

Its localities were usually in the northern and southern parts of the plot. The percentage of localities was highest in the ecotone (41%) and *T-C typicum* (29%), and smallest in *T-C stachyetosum* (Fig. 4K).

Puccinia arenariae (Schumacher) Winter

The fungus is a biotrophic species occurring exclusively in the telial stage on many representatives of the family Caryophyllaceae. It is reported as a common species both worldwide (Farr & Rossman 2010, over 600 records) and in Poland (Majewski & Ruszkiewicz-Michalska 2008).

Although the host was present in the entire study area, the fungus did not occur frequently. It was recorded at 41 localities, mostly at the lowest frequency (93% of records as very rare). The fungus was observed throughout the period of the plant's development, from April until November. Only a few records were noted initially (April to June). Their number increased in time and reached maximum in the second half of the vegetative season. A clear preference for *T-C typicum* was observed (almost 50% of localities) (Fig. 4J).

Hyphomycetes (Moniliales)

Alternaria alternata (Fries) Keissler

It is a ubiquitous and cosmopolitan species commonly occurring in different types of habitats worldwide (Farr & Rossman 2010, over 700 records). It is a saprotroph occurring on dead remains of many plant species and on other products of organic origin. However, it is often recorded as a pathogen infecting living plant tissues and causing mycoses in humans and animals. In all of Poland it is recorded on fewer than 50 plants (Anusiewicz 2008). It was not previously reported on *Stellaria holostea* L.

It was recorded on dying and dead host's leaves in the study area. Infected plants were collected from August until November but at only a few localities. It was recorded in each of the subcommunities and in the ecotone in a total of seven squares (Fig. 4T).

Botrytis cinerea Persoon *ex* Nocca & Balbis

Like the previous species, *Botrytis cinerea* is common worldwide and on a variety of substrates, living and dead (Farr & Rossman 2010, over 3000 records). In Poland it was recorded on fewer than 100 plant species, as a saprobic and as a pathogenic species (Anusiewicz 2008). It was not previously noted on *Stellaria holostea*.

The fungus was collected on dying leaf fragments in October but only at two localities in the southern part of the plot within *T-C typicum* (Fig. 4U).

Cladosporium herbarum (Persoon) Link

It is one of the most common species in the world (Crous *et al*. 2007; Farr & Rossman 2010, about 800 records). It also occurs commonly in Poland and is quite often recorded as a causal agent of mycoses and allergies in humans and animals. It was previously reported on *Stellaria holostea* but from few localities (Ruszkiewicz-Michalska & Mułenko 2008).

Cladosporium herbarum was collected on dying or dead plant organs (especially in autumn) but it was also recorded on living plant organs throughout the vegetative period. It was also recorded at single localities in the first two months of the vegetative season (April, May). The number of occurrences quite abruptly increased in June; the maximum values were recorded towards the end of the vegetative season when the plant's vegetative organs were dying.

The fungus occurred on nearly the entire research plot with the exception of two localities in its northwestern part (*T-C typicum*). The frequency of its occurrence was in the lower range of the scale (1, 2, 3), and it was recorded mostly as a rare species (44% of records) (Fig. 4C). The fungus cannot be treated as a pathogen posing a threat to

the development of the host population despite its frequent occurrence.

Fusarium avenaceum (Corda *ex* Fries) Saccardo

This is also a common representative of anamorphic fungi occurring worldwide on different substrates and in different habitats (Farr & Rossman 2010, over 300 records). In Poland it has been identified on *ca* 40 species of hosts (plants and fungi) and has been reported from very many localities. The fungus also causes diseases in humans and animals (Świderska-Burek 2008). It was not previously reported on *Stellaria holostea*.

Fusarium avenaceum was collected frequently in the study area on living and dying or dead organs of the host plant during almost the entire vegetative season. It was initially noted only at single localities. A small increase of the distribution began in August and reached maximum in the last month of the vegetative season (November). The fungus occurred in only two frequency classes and was recorded most frequently (over 90% of records) in the lowest class (very rare), infecting no more than 1% of the *Stellaria holostea* plants in individual squares. It does not pose a threat to the host population.

The distribution range of *Fusarium avenaceum* covers 60% of the study area (60 localities). A clear preference for *T-C typicum* was observed, with nearly 70% of the localities recorded in it (Fig. 4G).

Fusarium oxysporum Schlechtendal

Like the fungi reported above, *Fusarium oxysporum* is a cosmopolitan species occurring commonly worldwide on different substrates and in different habitat types (Farr & Rossman 2010, *ca* 650 records). In Poland it is known from fewer than 50 plant species and as a saprotroph widely distributed on organic remains in terrestrial and aquatic environments (Świderska-Burek 2008). It has also been recorded as a human pathogen. It was not previously reported from *Stellaria holostea* L.

The fungus was recorded at only one locality in the study area, in *T-C stachyetosum* in July (Fig. 4X). It was recorded on dying leaf fragments on one plant specimen.

Phacellium episphaerium (Desmazières) U. Braun

Phacellium episphaerium is a biotrophic parasite developing on living organs of plants, mostly on leaves, belonging to two genera: *Stellaria* and *Myosoton* (Caryophyllaceae). In a monograph (Braun 1998) it is reported as a frequent species, observed on 12 species of plants belonging to the genus *Stellaria* (incl. *Myosoton*) distributed in Asia, Europe, North Africa and North America. It has been reported on *Stellaria holostea* from only four European countries (Estonia, France, Great Britain, Poland). On the checklist of fungi in Poland it was noted at almost 20 localities, on five plant species: *Myosoton aquaticum* (L.) Moench, *Stellaria graminea* L., *S. holostea* L., *S. media* (L.) Vill., *S. nemorum* L. (Kozak 2008). The fungal databases give only three records and should be updated (Farr & Rossman 2010).

Phacelium episphaerium was one of the most frequently recorded species in the study area and was also observed throughout the vegetative season. It occurred on young plants as early as in May, at many localities at the same time (96% of total area). Its distribution was large over a long period of time (V–IX). The number of localities decreased considerably only at the end of the season (October) but it still occurred in almost 50% of the area. The fewest localities (only 16) were found in late autumn (November). Interestingly, the sporing structures of the fungus (coremia) are very persistent; they were observed on dead but undecomposed plant remains after wintering.

The fungus occurred very often in the study area but its frequency was in the lower range (1–3), most frequently in the first frequency class (over 40% of records) and least frequently in the third class. The fungus did not infect more than 30% of the plants occurring in individual squares.

Phacelium episphaerium is the only species recorded in the entire study area and its contribution in each of the subcommunities and the ecotone is 100% (Fig. 4B). However, it does not markedly influence the development of the

host plant as it causes only spot infections which do not result in a significant increase in the plant's infection rate.

Melanconiales

Colletotrichum dematium (Persoon *ex* Fries) Grove

The fungus is a cosmopolitan multi-host species recorded on many species of herbaceous and woody plants worldwide (Farr & Rossman 2010, over 300 records), known mostly as a saprobic species but sometimes also found on living plant organs. In the monograph on Coelomycetes, Sutton (1980) reported it on herbaceous and woody plants belonging to 118 genera from 37 countries. In Poland it was recorded on more than 30 species of host plants, and at numerous localities (Kozłowska & Wołczańska 2008). The species was not previously recorded on *Stellaria holostea*.

The fungus was collected on dying or already dead plant fragments in the study area. It occurred in the last three months of the vegetative season. It was recorded at 21 localities in a relatively short period of time and in the lowest frequency class. The highest number of records (15) is from October. The fungus occurred in *T-C stachyetosum* most frequently and in the transition zone least frequently (Fig. 4L).

Sphaeropsidales

Ascochyta anisomera Kabát & Bubák

Apiocarpella anisomera (Kabát & Bubák) Mel'nik

Ascochyta anisomera is a very rare fungal species. It is reported from only three localities in the fungal databases: one locality on *Stellaria nemorum* (Italy, south Tyrol), and two localities on *Myosoton aquaticum* (Czech Republic, Bohemia, Turnov town) (Farr & Rossman 2010). Vanev *et al*. (1997) also report it on the same plants from single localities in Bulgaria. In Poland it was recorded only on *Stellaria nemorum* at four localities: the Kotlina Zakopiańska basin (Sałata *et al*. 1993), Białowieża National Park (Mułenko 1996; Faliński & Mułenko 1997), Nowy Sącz and the Białowodzka Góra Reserve near Nowy Sącz (Romaszewska-Sałata *et al*. 1997). It was not previously reported on *Stellaria holostea*.

The fungus was observed very rarely in the present study. It was collected in October, at one locality in *T-C typicum*, on living plant leaves (Fig. 4W).

Ascochyta stellariae Fautner

This is another rare fungal species. In the fungal databases it is reported from only one locality on *Cerastium alpinum*, Poland, Western Carpathians, Babia Góra Mt. (Chlebicki 2002; Farr & Rossman 2010). However, it is also known from two other localities in Poland: the Durne Bagno Reserve in the Pojezierze Łęczyńsko-Włodawskie lake district (Mułenko 1988) and Białowieża National Park (Mułenko 1996), on *Stellaria holostea* in both cases. The species seems to be considerably more frequent and requires further investigation.

The fungus was collected in the study area very often throughout the vegetative season. It was identified on living as well as dying and dead fragments of plants from the same year and on remains of plants from the previous year (April). It first occurred on young plants in May, initially at a few localities (12). The number of records increased severalfold in June and remained at a similar high level until the end of the season (VI–X). A small decline was observed in July and August, and the number of records was highest in September (63). The distribution clearly decreased in November. The fungus was recorded 230 times at 95 localities during the study period.

The frequency of its occurrence in individual squares was not high, however, and fell within the lower range of the scale (rare and very rare), with records in the first class prevailing (over 90%). The fungus was not recorded at only five localities in the northwestern part of the study area (*T-C typicum*). It occurred commonly and its percentage contributions in individual plant subcommunities and the ecotone were similar (Fig. 4D).

Phoma exigua Desmazières var. ***exigua***

The fungus is a multi-host species, a weakness parasite or saprotroph, recorded worldwide

on many species of herbaceous and woody plants (Farr & Rossman 2010, *ca* 1120 records). In Poland it was also collected on numerous plant species, including *Stellaria holostea*, and at many localities (Ruszkiewicz-Michalska & Kozłowska 2008).

Phoma exigua var. *exigua* was not very frequent in the study area but the period of its occurrence was long. It was recorded in early spring (April) on plant remains from the previous year. It was not collected in May. The first infection symptoms and sporing structures initially occurred in June, mostly on lower, slowly dying leaves of a plant. The number of records at this time was not high but it increased successively (from 5 to 10) over the next two months, reaching maximum in September (18) after which it decreased again as the plant was dying (from 14 down to 9). The development of the fungus generally coincided with the second half of the vegetative season.

The number of infected plants in each square was small and did not exceed 10% of the plants. The fungus was recorded in the two lowest classes of the frequency scale, mostly in the first class (very rare, 95% of records). Its range of occurrence covered almost half of the study area (48 localities). The species did not exhibit clear preferences for any of the subcommunities, although proportionally the number of localities was highest in the ecotone (55%). The contributions to the subcommunities were very similar (47% in *T-C typicum,* 44% in *T-C stachyetosum*) (Fig. 4I).

Placosphaeria stellariae (Libert) Saccardo

The species is quite rarely collected. In the fungal databases it is reported only from Armenia on *Dianthus subulosus* Freyn & Conrath and *Dianthus* sp. (Simonyan 1981), and from three localities on *Dianthus monspessulans* L. (Belluno Town, Italy) and *Stellaria* sp. (Ardennes region and Rhenogovia, France) in the Saccardo database (Farr & Rossman 2010). It is also on the checklist of fungi of Ukraine (3 records, www.cybertruffle.org.uk). In Poland it was collected only at a few localities on *Stellaria holostea*: Dobrzyń (Wróblewski 1915), Białowieża Primeval Forest (Siemaszko 1923; Mułenko 1996; Faliński & Mułenko 1997), Durne Bagno Peat Bog Reserve and Jezioro Długie Lake Reserve in the Pojezierze Łęczyńsko-Włodawskie lake district (Mułenko 1988).

The species was also rare in the study area. It was observed during nearly the entire vegetative period but occurred at only few localities and usually in the lowest frequency class. It was recorded at a total of 14 localities.

The occurrence frequency was in the lower range of the scale (1–2), mostly in the first class (90% of records). It was recorded considerably less frequently in the transition zone than in the subcommunities (Fig. 4O).

Phyllosticta holosteae Allescher

It is a rare fungal species worldwide. It has only one record on *Stellaria holostea* in the Saccardo database: Germany, Franconia region in Bavaria (Farr & Rossman 2010). In Poland it is known on *Stellaria holostea* from Białowieża National Park (Mułenko 1996, 1998) and on *Stellaria uliginosa* from the J. Brudzieniec Reserve in the Pojezierze Łęczyńsko-Włodawskie lake district (Mułenko 1988). The fungus requires further, more thorough field studies and additional investigations.

The species was not too rare in the study area. It was collected at 23 localities and observed during nearly the entire vegetative season. It was recorded at single localities for most of the season, and its distribution increased slightly only in September (9 localities). The fungus was most frequently collected in *T-C typicum* (15 localities, 29% of the area occupied by the subcommunity), less frequently in the ecotone (5 localities, 23%) and in *T-C stachyetosum* (3 localities, 11%) (Fig. 4M).

Phyllosticta sp.

The fungus was recorded on lower, dying or dead leaf fragments. Pycnidia (60) 100–200 µm in diameter. Conidial spores olive, elliptical-ovate, 3–8 × 1.5–4µm. The fungus requires further field studies and detailed investigations.

The fungus was recorded over four months of the vegetative season (July to October) in the study area but was collected rarely, at single localities

and in the lowest frequency class. It occurred at a total of 11 localities, in all the subcommunities and in the ecotone (Fig. 4R).

Septoria stellariae Roberge *ex* Desmazières

The fungus is recorded fairly frequently worldwide (Farr & Rossman 2010, 32 records). It has been observed on *Myosoton aquaticum*, *Cerastium* sp. and on seven species of the genus *Stellaria*. In Poland it is also known from numerous localities and recorded on four species of the genus *Stellaria*, mostly on *S. media* (Wołczańska 2008).

Septoria stellariae was recorded very frequently (241 records) in the study area, mostly on dying or dead, less often living, plant leaves. The occurrence range comprised almost the entire research plot (93%), and the fungus was observed throughout the vegetative period. The first infection symptoms are visible as early as in May. The fungus occurred at a small number of localities (from 8 to 19) in the first four months but its range of occurrence covered 63–76% of the area in the autumn period (IX–X). The number of localities decreased in November but its distribution was still relatively high (54 species). It's proportional contribution in the subcommunities and in the ecotones was high and did not indicate clear preferences for any of them. The frequency of its occurrence was in the lowest range of the scale (1–2) despite a high number of records (Fig. 4E).

Stagonospora sp.

The fungus was collected on dead lower plant leaves. Pycnidia large, black, 150–250 µm in diameter. Conidial spores olive 4–13-celled, 24–60 × 4–6 µm. The occurrence of fungi belonging to the genus *Stagonospora* on *Stellaria holostea* was not previously recorded.

The fungus was recorded rarely in the study area and only in the lowest frequency class but over a relatively long period of time. It was observed at few localities throughout the vegetative season. It was recorded at a total of 11 localities mostly in the southern and central part of the plot, in both subcommunities and the ecotone (Fig. 4P).

Discussion

Twenty-two microfungi were identified on *Stellaria holostea* in an area of 1 ha within only one association (*Tilio-Carpinetum*). This is quite a high number, as 18 fungal species were previously recorded on *S. holostea* in Poland, and a total of 32 species on all representatives of the genus *Stellaria* (Mułenko *et al*. 2008). Brandenburger (1985), for example, reports 15 fungal species on species of the genus *Stellaria* from all of Europe.

Species new to Poland are especially noteworthy. These include *Didymella holosteae*, an exceptionally rare fungus previously known from two other localities worldwide. *Leptosphaeria* sp. is also interesting; as mentioned, no fungus having such characters has been described before, and it may be new not only to Poland but also to science. Two other fungi, *Phyllosticta* sp. and *Stagonospora* sp., also may be new species, especially *Stagonospora*, as fungi of this genus were not previously recorded on *Stellaria*.

The list of microfungi includes six species rare in Poland, that is, known from five localities at most. These include first of all *Leptosphaeria stellariae* reported only from one locality in Poland and exclusively from the Białowieża Primeval Forest, *Phyllosticta holosteae* (2 localities), *Ascochyta stellariae* (3), *Placosphaeria stellariae* (4), *Ascochyta anisomera* (4) and *Peronospora parva* (5).

Stellaria holostea is a new host species for several fungal species. *Ascochyta anisomera* in an interesting collection, noted on plants with very soft and delicate leaves (*Stellaria nemorum*, *Myosoton aquaticum*). A few species classified as ubiquitous were also collected on *S. holostea* for the first time: *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum dematium*, *Fusarium avenaceum* and *Fusarium oxysporum*.

The species recorded here were reported in our previous studies on fungi occurring on *Stellaria*, but without a detailed analysis of their occurrence and distribution. In this study we list them as interesting floristic records for the first time.

Other analyses can be made as part of very intensive and detailed long-term studies. On the

one hand, the list includes species that are very common but were recorded only sporadically in the study area. Species such as *Fusarium oxysporum* (1 record), *Botrytis cinerea* (2) and *Alternaria alternata* (7) occurred on *Stellaria holostea* very rarely. The finding of their rare occurrence may be due to the fact that completely dead plant fragments were not examined in detail, or that *S. holostea* is rarely colonized by these species. As shown above, it is a new host for some of them. On the other hand, some very rare species known in Poland and worldwide from only a few localities were found to be relatively frequent in this work. In these investigations we observed their permanent presence in the forest environment, as constant and not ephemeral elements. Although usually with low frequency, these fungi were recorded during the entire vegetative season in each year of the study. These fungi include *Ascochyta stellariae* (Fig. 4D), *Peronospora parva* (Fig. 4F), *Didymella holosteae* (Fig. 4H) and *Placosphaeria stellariae* (Fig. 4O). We can also confirm that *Mycosphaerella isariophora* is indeed a very frequent species (Fig. 4A).

During our systematic studies over a period of several years we observed considerable temporal and spatial fluctuations of the occurrence of fungi. For instance, a long period of occurrence was not always related to an increase in the distribution of an organism. Of seven fungal species recorded throughout the vegetative season, only four occurred much more frequently and were recorded over almost the entire area (e.g., *Phacellium episphaerium*, 100 localities; *Mycosphaerella isariophora* and *Cladosporium herbarium*, 98; *Ascochyta stellariae*, 95). These fungi differ significantly in their occurrence frequency in individual squares and the entire area. Other fungi with a comparable period of occurrence did not reach maximum distribution. For instance, rust fungi (*Melampsorella caryophyllacearum*, *Puccinia arenariae*) were present throughout the vegetative season but the former was recorded 40 times at 27 localities and the latter 58 times at 41 localities.

The fungi recorded in the study can be classified into three ecological groups based on the type of plant colonization and the occurrence period. Some of them are biotrophic species growing on living organs of *Stellaria holostea* and are typical parasites of it. These are two rust species (*Puccinia arenariae*, *Melampsorella caryophyllacearum*), one species of downy mildew (*Peronospora parva*) and an anamorphic fungus (*Phacelium episphaerium*). Facultative saprotrophs which begin their development on still-living plant organs and continue on dead parts as typical saprotrophs form a larger group of fungi. They include some ascomycete species (Ascomycota, e.g., *Mycosphaerella*, *Leptosphaeria*) or anamorphic fungi (e.g., *Septoria*, *Phyllosticta*, *Ascochyta*). The third group comprises facultative parasites that live first of all as saprotrophs but also infect living plant organs. It is the most numerous group, comprising primarily anamorphic fungi belonging to genera such as *Alternaria*, *Botrytis*, *Cladosporium*, *Fusarium* or *Colletotrichum*. Their teleomorphic stages develop on dead plant organs, often only after wintering. The two latter groups are often included in so-called weakness parasites which attack weakened plants or their dying organs.

The results clearly show the need for further detailed research on individual fungal species. Investigations of this type on small permanent research plots provide an opportunity to collect more detailed data on the contribution and constancy of a specific organism in an environment.

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