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A review of the methods used for studies on parasitic fungi in natural plant communities

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The purpose of this paper is to reconsider the methods used so far and to propose standardization of studies on the microparasitic fungi in natural plant communities. The method should meet the following requirements: demonstration of the relationships between the pathogen and host, application in different environmental conditions, enabling repetition of studies at different times, giving comparable results.

The object of the discussion is to search for such study procedures which give a total information about the occurrence of phytopathological fungi and their hosts in natural plant communities. This concerns, among other things, the following problems: estimation of the frequency of fungi, their distribution, the infection degree of their hosts as well as analysis of the occurrence of fungi in relation to plant distribution. The degree of plant density is an important factor determining the appearance of the disease, and its evaluation should be an inseparable element in analysis of parasitism.

The mycological-ecological studies, using permanent study areas seems to be indispensable. Research plots need not be literally permanent. Here, a system of permanent, precisely localized points can be used which allow each time reconstruction of areas determined earlier. The most essential element of such studies is to provide for oneself (and future researchers) repeatability of studies, i.e. return to the same places and taking samples from the same areas.

Key words: parasitic fungi, host plants, ecology, plant communities, methodology.

INTRODUCTION

In the last time the rapidly progressing fragmentation and degeneration of natural ecosystems as well as increasing threat to them caused by pathogenic agents was observed (H o l m e s 1996). Therefore, the parasitic flora of natural plant complexes, especially the associations of fungi with plants in natural communities, as well as the rapid changes occurring in mycoflora and in the system of natural relations between the host and pathogen must be known better. Plant pathogens are one of more important elements of the natural environment (R a y n e r 1995) and they can be considered as indicators of the changes occurring in it (H i r s c h and B r a u n 1992).

In studies on the occurrence of fungal microparasites in natural communities there are still needed reliable research methods as well as comparative materials which can be helpful in observing the changes mentioned. The following elements distinguishing mycoflora seem to be essential here: taxonomic structure, floristic diversity, degree of naturalness, and way of occurrence.

SUBJECT AND RANGE OF PROPOSED ANALYSIS

Two kinds of studies on pathogenic fungi of vascular plants should be taken into consideration: mycological and phytopathological.

Mycology is a wide branch of science characterised by investigations into the floristic richness and role of fungi. Mycological investigations, also defined by the term "pure mycology", are experimental or theoretical, the aim of which is to acquire some new knowledge, in substance without practical application (T r i n c i 1995). The object of phytopathological studies is the plat and their main purpose is to learn plant diseases and control them. Phytopathological studies, also called "applied mycology" concern practical application of the acquired knowledge (T r i n c i l.c.).

The basic techniques used in phytopathological studies (in research plots and monocultures) have been properly developed and are well known. As monoculture is an artificial, homogeneous (frequently also genetically), considerably isolated system being constantly controlled by its utilizer, it can be relatively easily modified and adapted to new requirements. Intensive experimental studies on the differentiation of taxonomical forms, their pathogenicity and importance for man concern above all taxons significant from the economic point of view. This refers both to agricultural cultures and forest pathology (C a n n o n and H a w k s w o r t h 1995).

There are three information sources of empirical data in phytopathology (S a v a r y et al. 1995):

- quantitative estimation (often uses 5-grade scale: 0 – no disease, 1 – frequency below 1%, 2 – 1-5%, 3 – 6-25%, 4 – 51-100% frequency);
- types of pathologic reactions (e.g. 4-grade scale: very sensitive, sensitive, resistant, very resistant);
- quantitative measurements of infection effectiveness (i.e. proportion in the composition of spores inducing diseases), intensity of bearing spores (production of spores by the population of the pathogen), and inoculation period (measurement of the time between infection and sporulation); the issues of this point can be studied only in greenhouse cultures.

The methods for estimation of the occurrence of microparasitic fungi in natural environment aim at obtaining the same or approximate information groups. With regard to fungi in natural communities, neither the methods specific for phytopathology nor those used in studies on macromycetes, higher plants, mosses, liverworts and animals can be applied (Majewski 1971; Dinoor and Eshed 1984; Burdon 1987; Chlebicki 1989; Carroll and Wicklow 1992; Wenström 1994; Watling 1995; Mułenko and Majewski 1996). Using the same methods is impeded, among other things, by the mosaic structure of natural plant communities, varying density of populations, a big genetical diversity of plants and fungi, physiological differences resulting from adaptational behaviours, differentiated influence of the environment and competition. The latter factor applies both to plants and pathogen communities occurring on particular hosts. With the elapse of the vegetation season the estimation becomes more and more difficult. New species appear successively, the degree of infection as well as the variety of species of fungi populating plants increase.

One of the main estimation difficulties is also the fact that most microscopic fungi cannot be identified in the field or their frequency determined accurately from induced, most often little specific pathological symptoms. The basic principle of the research work is to collect each time plants with infection symptoms, and rich herbarian materials should be available for analyses.

The used methods must take into account the fact that the way of occurrence of fungi in relation to their distribution on host plants in natural communities differs from that in monoculture. The distribution of parasites is generally limited only to a part of the area occupied by the host (Durrieu 1966; Majewski 1971, 1978), whereas the occurrence intensity of diseases, change in predomination within taxonomical groups and their distribution undergo significant alterations in time and space. This problem has not been studied as yet exactly in natural conditions. The taxonomic structure of mycoflora – the basic object of the present analyses – does not give information about environmental and spatial relatioships of these phenomena.

In mycological studies carried out in natural plant communities, one of the basic problems is adaptation of an appropriate method for quantitative determination (frequency) of the occurrence of fungi. The method should meet the following requirements:

- demonstration of the relationships between the pathogen and host,
- application in different environmental conditions,
- enabling repetition of studies at different times, giving comparable results.

The discussion of the research methods presented below concerns such studies which show the whole parasitic flora in a definite area.

CHARACTERISTICS OF THE PRESENT RESEARCH TECHNIQUES

General remarks

Mycological studies, as those in inductive sciences, are based on sampling, i.e. drawing conclusions from a certain number of samples which represent a community (e.g. all species of fungi in a forest, physiographic object, region and the like). The indispensable number of samples, their magnitude, the way of spatial distribution and similar principles are generally determined by the sampling theory (F a l i ń s k i, msc.). Detailed sampling principles result depend among other things on the purposes and standards adopted in the given field of studies (mycology, lichenology, bryology and the like) or the direction of studies (e.g. in structural, dynamic, chorological studies).

The specificity of the research object may require sticking to accurate sampling time, sampling repeated at definite intervals and the like. The latter requirement can be met most easily by forming a system of permanent research areas (F a l i ń s k i, l.c.).

Studies in large areas

In the first analyses of the occurrence of fungal micropathogens in natural plant communities the method of field studies consisted in simple attributing the collected fungi to definite plant communities. Further papers concerning this problem were accomplished by: M a j e w s k i (1967, 1971), K u ć m i e r z (1973), R o m a s z e w s k a–S a ł a t a (1977), D a n i l k i e w i c z (1987), C h l e b i c k i (1989). The frequency of fungi was not estimated or it was presented by description and with little accuracy.

Studies by using the method determining the frequency of parasitic fungi in communities were carried out for the first time by K u ć m i e r z (1977). In these studies the author determined: (I) the frequency of fungi in plant communities, using a 5-grade scale: 1 – fungi occurring sporadically, 2 – rarely (2-10% of infected plants), 3 – relatively frequently (11-30%), 4 – frequently (31-60%), 5 – massively (> 60%); (II) degree of pathogen induced infection of plant (degree of infection); here he used a 4-grade scale: a – traces of infection, b – weak infection (2-20% of infected plant surface), c – medium infection (21-60%), d – strong infection (> 60%).

The frequency was determined approximately and referred directly to the community or – when it occupied a larger area – small research plots of 25 to 60 m^2 were chosen randomly. For the whole community the mean of all records over the year was calculated, and then the mean of the whole 3-year study period.

A similar method was also used by M u ł e n k o (1988a,b) when studying the parasitic flora of the Łęczna-Włodawa Lake District. However, the first pilot studies (M u ł e n k o 1981) showed that the use of an exact scale (e.g. a 5-grade one) caused divergence and inconsistency between the frequency determined in selected areas and that referred to the whole community. In both cases the author (M u ł e n k o l.c.) used a 3-grade scale: 1 – sporadically (single infected plants were observed), 2 – quite often (up to 10 infected plants), 3 – commonly (a large number, sometimes of strongly infected plants were observed).

Among the studies of this period a special attention should be given those carried out by B r a u n (1982). He omitted the floristic aspect of studies and concentrated on associations of fungi with plant communities. The method of his studies was similar to that of those mentioned above. In communities occupying a larger area, he determined the frequency of fungi in areas of 100 m^2. He did not estimate the infection degree of plants. In his studies two basic kinds of frequency were distinguished:

1) *absolute frequency – the number of infected plants in the community, depending on the density of plants*;
2) *relative frequency – referring to the number of noninfected plants in the community independent of their abundance.*

In direct determination of the amount of fungi B r a u n (1982) used an estimate, 7-grade scale denoted by a combination of marks „+" and „–":

++ = *severe, dominant occurrence in the phytocoenosis, relative as well as absolute large frequency, often more than 30% of the hosts infected*;
\+ = *occurrence moderate, often scattered, incomplete, always less than 30% of hosts infected, relative frequency also moderate*;
+(+) = *the same occurrence as in „+", but relative frequency large (host not very frequent in the studied phytocoenosis)*;
+– = *absolute as well as relatively small frequency, always less than 5% of the hosts infected*;
+(–) = *absolute frequency small, but relative frequency large*;
–(+) = *single occurrence (host abundant in the phytocoenosis)*;
–(–) = *single occurrence (host rare in the phytocoenosis).*

In the estimation scale he distinguished 4 categories of so-called particular frequency figure (PFF):

++ = **4**
\+ and +(+) = **3**
+– and +(–) = **2**
–(+) and –(–) = **1**

Using these four categories he calculated for each species so-called mean frequency figure (MFF) (B r a u n 1982; H i r s c h and B r a u n 1992):

$$MFF = \frac{\sum PFF}{NP}$$

MFF – mean frequency figure, PFF – particular frequency figure, NP – number of investigated places.

In the final stage of analysis, for fungi found in the particular communities three categories of mean frequency figure (MFF) were distinguished by the author (B r a u n l.c.):

Cat. I: MFF > 1 (–4), parasitic fungi very frequent, characteristic and dominant in the plant community;

Cat. II: MFF 0.5 – 1, parasites regularly occurring in the plant community, but not frequent and not dominant;

Cat. III: MFF < 0.5, parasites only occasionally occurring in the plant community.

The papers presented above close a period of investigations conducted in large areas. Besides positive elements concerning floristic values and their common features, there are some shortcomings, particularly of methodological character, e.g.: lack of univocal and uniform determination of the frequency of fungi, and in most cases there is lack of information about the number of the studied localities, as well as about their plants (data about plants come from literature).

Studies in permanent research plots

Such studies were carried out for the first time in the north-western states of the USA, Washington and Idaho, in 1946-1948 (C o o k e 1955). Eighteen plots (each of about 100-150 m^2), situated in 6 different plant communities, were studied. Each plot was studied 3 times in different periods of the vegetation season. The frequency of the occurrence of the collected organisms was not estimated, nor the number (for comparison) of the plants was given. The main purpose of his studies was tabular listing of the collected cryptogamic plants of different taxonomic groups. The significance of these studies, as of those mentioned at the beginning of the chapter, is largely floristic.

The studies carried out in the Białowieża National Park in 1987-1991 (program "Crypto") can be included into ecological ones (F a l i ń s k i and M u ł e n k o 1992, 1995, 1996). They comprised one forest compartment about 144 ha in area, divided into 144 one-hectare squares (permanent plot V-100 BSG UW). In this area 6 most important communities of primeval forest were found.

The occurrence of fungi like that of all other cryptogamic plants was estimated in a 3-grade scale: rare, frequent, common (M u ł e n k o 1995a; F a l i ń s k i 1996). After carrying out introductory, one-seasonal pilot studies, the use of a more exact scale in 1 ha area appeared ineffective. In this scale fungal micropathogens occurred: rarely (1-10% of infected plants), frequently (11-30%), commonly (31-60%). In 1-ha area infection on a mass scale (over 60%) was not observed (M u ł e n k o 1995b; M u ł e n k o and M a j e w s k i 1996). At the some time by using the same methods, studies on the occurrence of potential plant hosts of parasites were carried out (G ł o w a c k i and Z a ł u s k i 1995).

During studies on the occurrence of phytopathogenic fungi in the Caledonian pine forest in Scotland, the author (M u ł e n k o, in press) used a similar 3-grade evaluation scale of frequency. Fungi were collected from 133 research plots of 25-30 m^2, distributed in an area of about 200 ha. Unfortunately, the frequency of plant occurrence was not estimated.

In 1992-1995 studies were conducted which resulted in obtaining better results. For them a permanent 1 ha plot was chosen in the area of Białowieża National Park (areas of the International Biological Programme No 40 BSG UW; F a l i ń s k i 1966). The area comprises one forest community (*Tilio-Carpinetum*) occurring in three subcommunities and in 6 variants. The area was divided into 100 squares of 100 m^2 each. The studies were conducted according to the following scheme:

(1) – The first year was devoted to pilot studies which included: general determination of their method, denoting the basic species of fungi on host plants, presentation of the estimation way of the frequency of fungi, preparation of the lists of plants in each square and estimation of the cover degree.

(2+3) – In the two following years the occurrence of parasitic fungi was consequently observed and materials were collected over the whole vegetation season from April to November. Observations in each square were carried out every other week. Every square was penetrated at least 16 times.

(4) – The fourth year was devoted to supplementary studies, listing of plots, measurements of the illumination of the forest floor, preparation of the computer data-base and analysis of all fungi on two selected plant species (*Anemone nemorosa* and *Stellaria holostea*).

Estimating the occurrence of fungi a small modification of the 5-grade scale proposed earlier by K u ć m i e r z (1977) was made:

1 – sporadical ($\leqslant$ 1%, single infected plants),
2 – rare (2-10% of infected plants),
3 – frequent (11-30%),
4 – common (31-60%),
5 – in mass (> 60%).

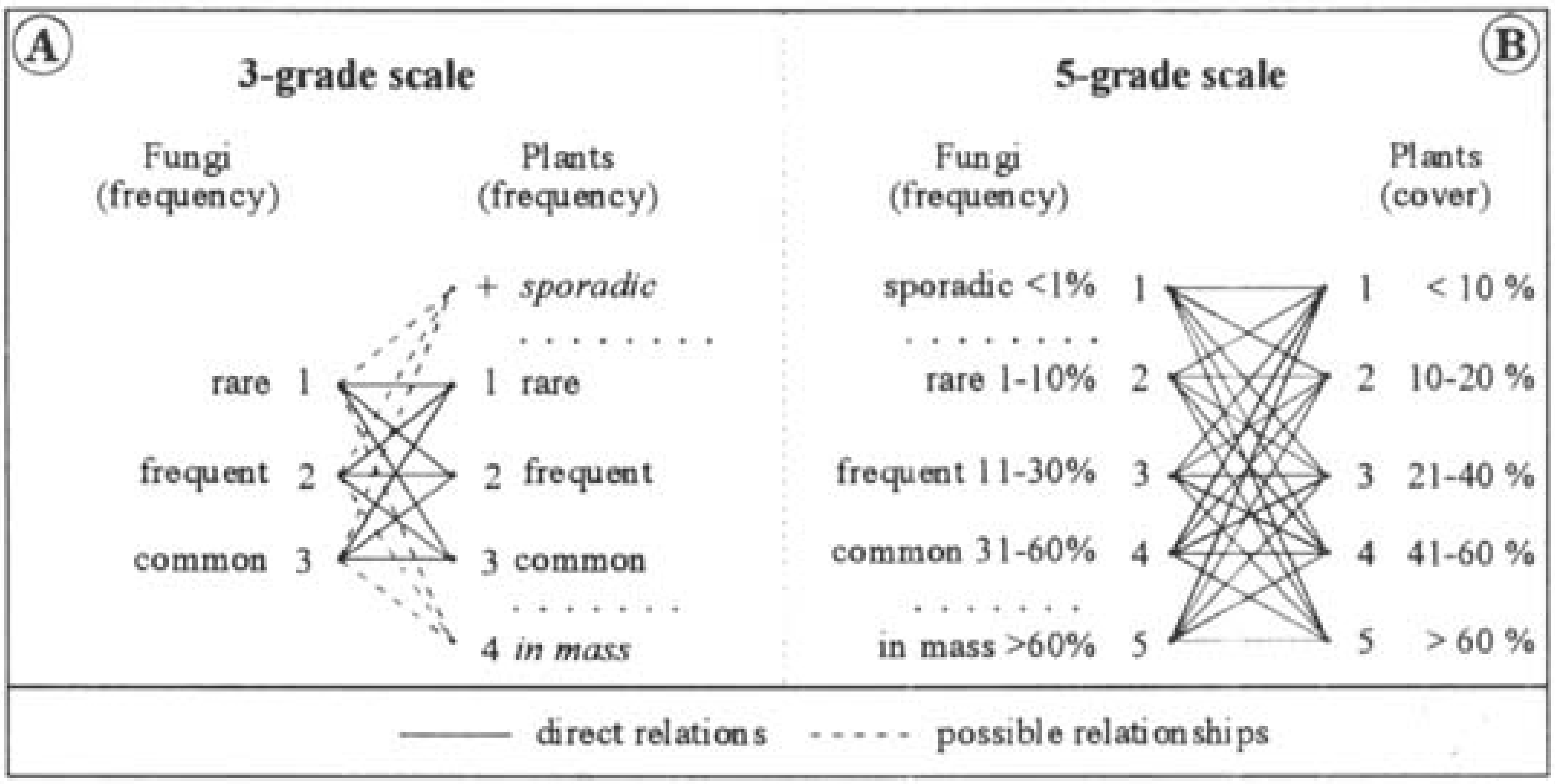

Fig. 1. Number of possible combinations between frequencies of hosts and pathogens

In the research area the frequency of fungi was estimated first of all according to the 3-grade scale (2, 3, and 4). Two extreme cases (1 and 5) were rarely observed. Minimal frequency (sporadically) was adopted also for the fungi whose presence was observed only in the laboratory.

Estimation of the frequency of host plants was made by using the comparable 5-degree scale (Fig. 1). This made possible analysis of the distribution of fungi in relation to the occurrence of plants.

DISCUSSION OF THE RESEARCH METHODS USED SO FAR

Studies on the occurrence and role of fungi in plant communities have been carried out in various ways, and quantitative estimation of the occurrence of parasitic fungi has caused the researchers many difficulties. An estimation method has been generally used to determine the frequency of fungi. Estimation is the basic method to determine the number of fungi in such studies in which exact measurements are impossible, e.g. when the studied phenomena are characterized by successive changes in time and space, and also when difficulties appear in determining the number, cover, density, distribution of plants and the relationships between the study objects (F a l i ń s k i, msc.). A correctly developed estimation scale should present in a simple way and precisely the variation scale of the given phenomenon and, if needed, make possible the transfer of a given object to other classes (F a l i ń s k i l.c.). In mycological-ecological studies concerning the occurrence of microparasites in natural plant communities estimate assessment seems to be the basic method.

The first papers were of floristic character and limited to determination of the specific composition of fungi in particular research plots. These studies resulted in lists of the collected species of fungi and their numbers estimated in the individual communities. Among these papers the first extensive characteristics of parasitic mycoflora was given by M a j e w s k i (1971). In his paper he presented among other things: an analysis of mycoflora similarity of the particular communities, the participation of taxonomical groups of fungi and seasonal variation reckoned on the basis of so-called herbarian samples. He specified the amounts and lists of exclusive species for every community, confirming floristic and habitat differences of the communities investigated. He compared the proportions between the number of the collected species of fungi and that of the plants forming the particular communities. He laid special emphasis on demonstrating the floristic variety and differences in the quantitative composition. From a carefully prepared index of the collected fungi the distribution of fungi in the particular compartments of the Białowieża Primeval Forest can be shown, which makes possible comparison of the results obtained by M a j e w s k i (l.c.) with those of studies carried out by other methods, e.g. by using permanent research plots.

The above presented characterization of the occurrence of micropathogens in communities has been adopted as standard and is used in all papers concerning the ecology of parasitic fungi.

Among the Polish authors, K u ć m i e r z (1977) used a most detailed, 5-grade scale to estimate the frequency of phytopathogenic fungi. However, this author (l.c.) seems to have not taken advantage of the possibility to interpret the results, rendered him by the method used. He referred the estimation of the frequency only to fungi, and their association with plant communities was analysed on floristic level in the way similar to that in other papers (see above). Neither did the author analyse the degree of plants infections estimated by himself. Both estimation types – frequency and infection degree – were listed only in tables. However, from the to date and later studies it appears that this problem should be subject of independent and very carefully prepared studies (compare B u r d o n 1987; L i n d e r s et al. 1996; W e n n s t r ö m and E r i c s o n 1994; W e n n s t r ö m et al. 1995; C a r l s s o n et al. 1990; B u r d o n and J a r o s z 1992).

B r a u n (1982) made an attempt at using a new method for showing the relationships between parasitic fungi and plants communities. However, the way of his distinction of two basic types of frequency: relative frequency and absolute frequency is controversial. Both frequency types were characterized not precisely and they do not refer to any defined way (e.g. scale) of estimating the density of plants and fungi. They are utilized as the basic value in 7-grade scale used for estimation of the frequency of fungi in field investigations.

From the practical point of view controversial is also the developed and relatively complicated 7-grade scale for estimation of frequency. A higher scale increases the probability of making mistakes and thereby decreases the possibility to compare the investigation results. The probability of erroneous estimation increases when studies are conducted in conditions of exceptionally mosaic and floristically rich communities (e.g. ruderal and synanthropic). From the author's own experiences it results that even in a 100 m^2 area — if it is rich in plant species or is characterized by high density of plants — the estimation of the frequency of fungi in a scale higher than 5-grade becomes very difficult.

The results of final analysis obtained by B r a u n (1982) diverted considerably from those expected. In several cases the mycological characteristics of some communities were compatible, and the same species of fungi distinguished different plant communities. A strong infiltration of species between different phytocoenoses was observed, e.g. 75% of parasitic fungi found in agrophytocoenoses, belonging to distinguished categories (I and II), occurred also in neighbouring meadow and ruderal communities (H i r s c h and B r a u n 1992).

For several reasons the studies of B r a u n (1982) attract attention: (a) for the first time, though with small precision, the occurrence of fungi was estimated in relation to plant density in communities; (b) they were the first attempt at analysing the frequency of parasitic fungi to find species distinguishing plant communities; (c) the obtained results seems to indicate that, despite the fact that pathogenic fungi are a permanent component of every community, their diagnostic value is statistically insignificant.

Parasites, like other groups of fungi, e.g. lignicolous fungi (B u j a k i e w i c z et al. 1995) are above all associated with the substrate on which they occur, i.e. the living host plant. However, the environmental conditions formed by a given community determine: (1) occurrence of pathogens in it, (2) the degree of plant infections, (3) occurrence intensity (frequency) of pathogens, (4) their distribution, (5) occurrence of the members of individual taxonomic groups and the conditions of their predominance.

In the studies carried out so far not all of the above five points were taken into consideration and analysed. Field studies always give an answer to point one (C l a y 1995). The degree of plant infections (point 2) by some species of parasites can be easily determined in the field, but most often laboratory studies are required. Its precise estimation in a large research area is rather impossible.

An answer to point 4 and 5 can be given by the results of the studies carried out in permanent research plots. The exactness of studying the distribution of fungi depends on the size of the permanent research plot (point 4). Mycoflora can be studied more precisely in a small area; besides, the precision can be only approximate. A comprehensive mycological characteristics of a definite area cannot be obtained even after 25 years of studies (H a w k s w o r t h 1991; C a n n o n and H a w k s w o r t h 1995). Information about the distribution degree of fungi is the basic source for determination of their preference with regard to communities, terrain forms and substrates. In "Crypto" studies the preferences were determined in relation both to single species and whole systematic groups (M u ł e n k o 1995b; M u ł e n k o and M a j e w s k i 1996).

In all author's studies mentioned above it was demonstrated that in natural conditions of forest communities imperfect parasitic fungi predominate (ref. point 5). Predomination of fungi of this group is evident both in regard to the floristic richness (defined by taxonomic structure) and distribution (defined by the spatial structure) (Fig. 2). The answer to point 3, i.e. determination of the frequency of parasitic fungi in field studies is still an important problem (see below).

PROPOSITIONS OF STANDARIZATION OF RESEARCH METHODS IN THE FIELD

The object of this discussion is to search for such study procedures which give a total information about the occurrence of phytopathological fungi and their hosts in natural plant communities. This concerns the following problems: estimation of the frequency of fungi, their distribution, the infection degree of their hosts as well as analysis of the occurrence of fungi in relation to plant distribution.

Estimation of the frequency of fungi

In most studies carried out to date a 3-grade scale (Fig. 1A) has been used to determine the frequency of fungi. It was used both in studies conducted in large areas (e.g. M u ł e n k o 1988 a,b; R o m a s z e w s k a-S a ł a t a 1977) as well as in a permanent large area of 144 ha (programme "Crypto"; F a l i ń s k i and M u ł e n k o 1995; M u ł e n k o 1995a). The 3-grade method is the simplest and also most universal. In any environmental conditions it allows estimation of three basic and also the most important types of frequency – **minimal**, **medium** and **maximal**.

This scale has been extended to 5-grade one by two extreme, contrasting categories: sporadic (1) and mass (5) occurrence. The main stress of the frequency in the 5-grade scale is laid on values specific for 3-grade one (Fig. 1B). Sporadic (frequency 1) and mass (frequency 5) occurrence can be relatively easily observed in the field. In natural conditions these two types of frequency were rarely observed (Fig. 2). The biggest role is played by records in which the frequency of fungi has been defined as rare (frequency 2). Fungi occurring at a higher frequency, e.g. frequently (3) or commonly (4) were analysed much more rarely. This rule refers both to the whole mycoflora found in the research areas mentioned and to fungi populating single plant species (Fig. 2).

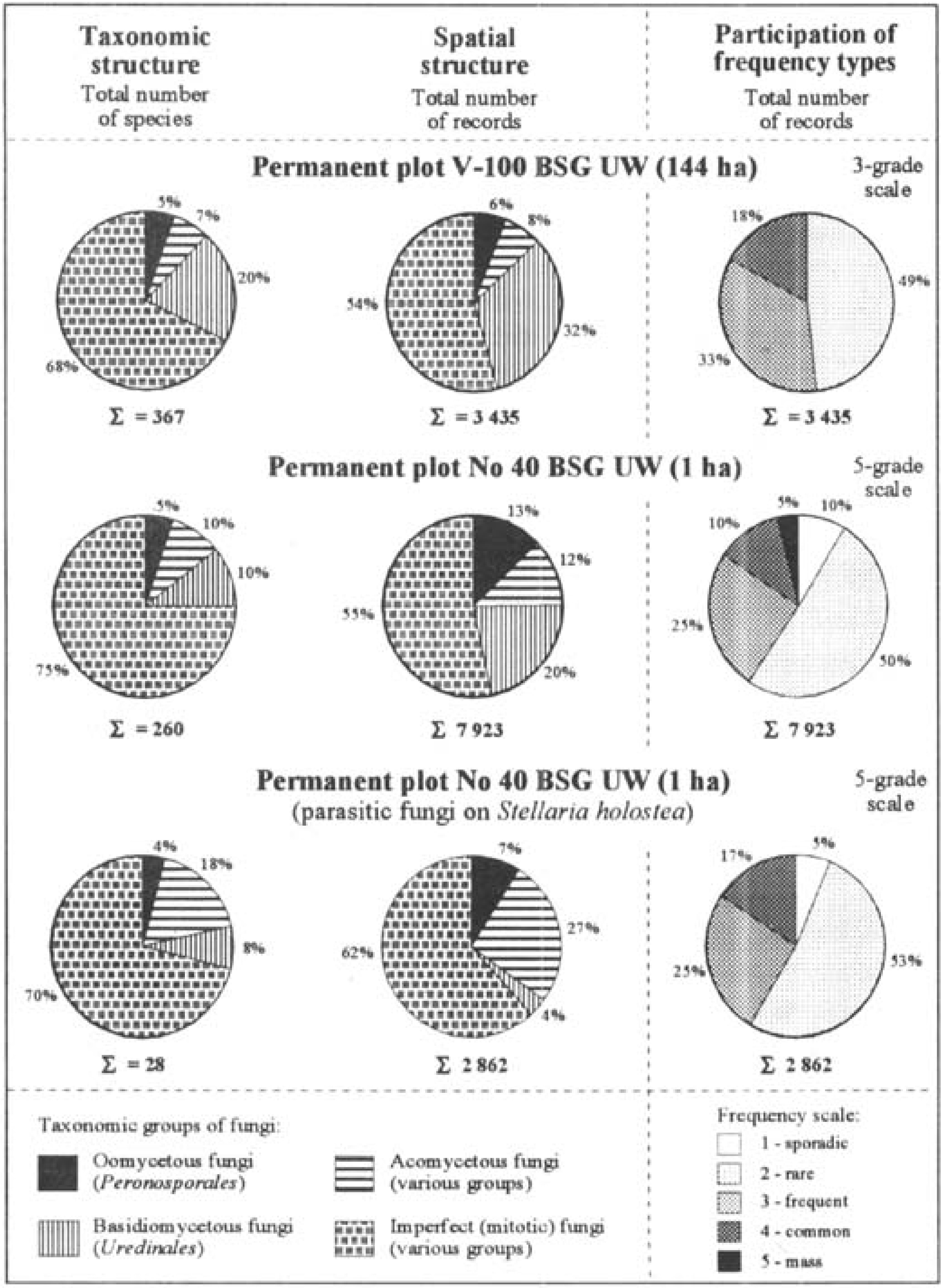

Fig. 2. Comparison of taxonomic and spatial structure of parasitic mycoflora

The most effective estimation of the frequency of fungi in natural conditions seems to be done by introducing the following rules: 1) use of the 3-grade scale in areas of about 1 ha or larger; 2) use of the 5-grade scale in areas from 25 m^2 to 200-400 m^2; 3) exact counting of all (healthy and affected) plants in areas below 25 m^2.

Only in the latter case a more accurate estimation of the degree of plant infection is possible. The area involved should enable an exact (not estimate) determination of the numbers of host plants. In such cases areas not exceeding 1 m^2 are usually used (compare, e.g. Linders et al. 1996; Wennström and Ericson 1994; Wennström et al. 1995; Carlsson et al. 1990; Burdon and Jarosz 1992). An exact analysis of the age and space structure of the studied plant populations is also required.

Estimation of plant frequency

The presence of the host plant is an indispensable condition for detection of a pathogen. Both mentioned organisms form one compact complex subjected to the influence of various environmental factors. The degree of plant density is a very important factor determining the appearance of the disease, and its evaluation should be an inseparable element in analysis of parasitism (e.g. Burdon 1987, 1992).

In to date mycological studies the frequency of host plants has been determined only twice. For the first time – in a descriptive way – it was done by Braun (1982). In "Crypto" programme the 3-grade scale was adopted (Mułenko 1995a; Faliński 1996).

In the area No 40, the author (Mułenko, msc.) estimated the frequency by a 10-grade scale. In its final version this scale was a little modified, in that only 5 frequency degrees were distinguished (Fig. 1B). The first point of this scale, concerning a plant cover smaller than 10%, is relatively important. In the studied area plants predominated considerably. Only one degree (5 – mass) was adopted for determination of the group of plants occurring at a frequency over 60%; this plant group was the least numerous.

In permanent but not quite large study areas the use of the 5-grade scale seems to be optimal, or appropriate modification of the 10-grade scale. For studies in large areas the safest is the 3-grade method.

Joint analysis of the frequency of host/pathogen system

Both the frequency of host plants and particularly that of their parasites have been determined several times, but they have not been analysed. As a matter of fact, the following questions and others have not been answered yet: (a) What types of host plant/parasite associations occur most frequently?

(b) What frequency type of parasites predominates within a specified type of plant density? (c) At what plant density is a parasite infection most intensified?

The method of comparable determination of the frequency of host plants and their parasites makes possible analysis of a relatively large number of their associations, the result of which can be detailed demonstration of the dependencies occurring in natural habitats (Fig. 1). For example, by the 3-grade scale fungi of a low frequency (1 – rarely) can be analysed in regard to: 1 – rare, 2 – frequent or 3 – common occurrence of plants. Using this scale we can analyse as many as 9 different combination types in the host/pathogen system (see also Fig. 1A):

1/1, **1**/2, **1**/3 **2**/1, **2**/2, **2**/3 **3**/1, **3**/2, **3**/3

bold numerals – fungal frequency, normal numerals – plant frequency

It is also possible to use another combination in which fungi are estimated by the 3-grade scale, and plants by the 5-grade one (Fig. 1A). The number of possible combinations increases here to 15.

In the 5-grade scale the theoretical number of combinations between the frequencies of host plants and pathogens is 25 (Fig. 1B). On the basis of this scale an attempt was made to analyse the occurrence of fungi found on *Stellaria holostea* (Fig. 3), in relation to the distribution of this plant in an area of 1 ha. On this host 22 parasite species were collected, which were recorded at 2.862 stands. The association of pathogenic fungi with plant hosts was analysed in two systems: fungi/plants and plants/fungi (Fig. 4).

System: fungi/plants. In the whole mycoflora species occurred most frequently at frequency 2 (rare). Their high amount (about 60%) was found in all distinguished types of plant density (Fig. 4A). Fungi of frequency 3 and 4 occurred much more rarely. Fungi of frequency below 1% occurred in very small amounts. The occurrence of any species on a mass scale (> 60%) was not recorded. In every frequency interval the amount of fungi was on a comparable level.

Among the distinguished fungi of a small frequency (2) only *Peronospora parva* occurred. In the case of *Phacelium episphaerium* the amounts of frequency 2 and 3 were comparable. *Mycosphaerella silenicola* occurred largely at high intensity (4).

System: plants/fungi. The basic information presented in this version of analysis is identical with the previous. However, more distinct is amount of fungi in different forms of plant density (Fig. 4B). In analogous global listing it can be seen more clearly that the subsequent types of frequency (rare, frequent, common) show a distinct tendency to decrease. In the occurrence

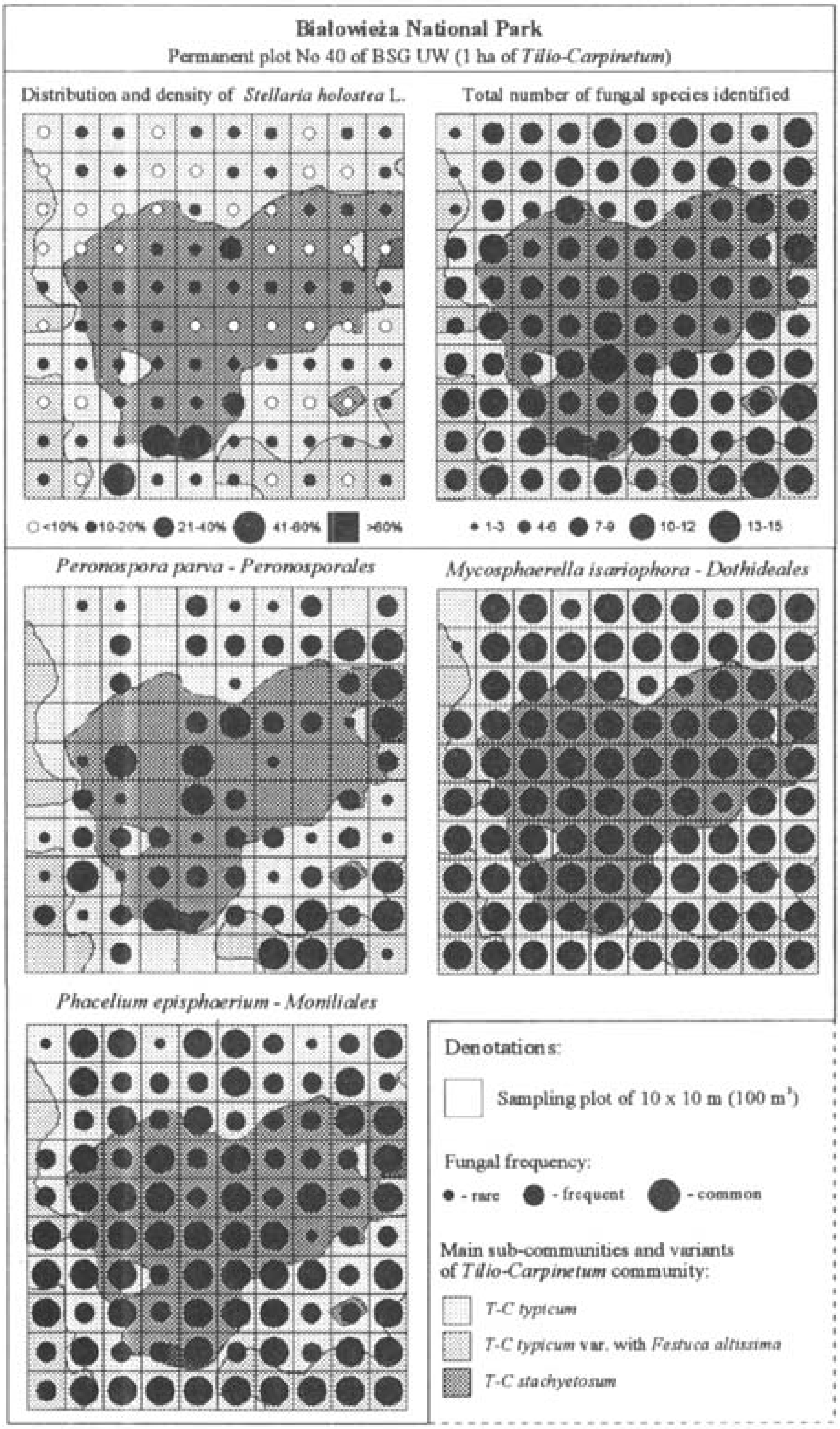

Fig. 3. Density of *Stellaria holostea*, its parasitic mycoflora and some fungal species collected on the host plant (generalization of all data recorded) (origin. elab.)

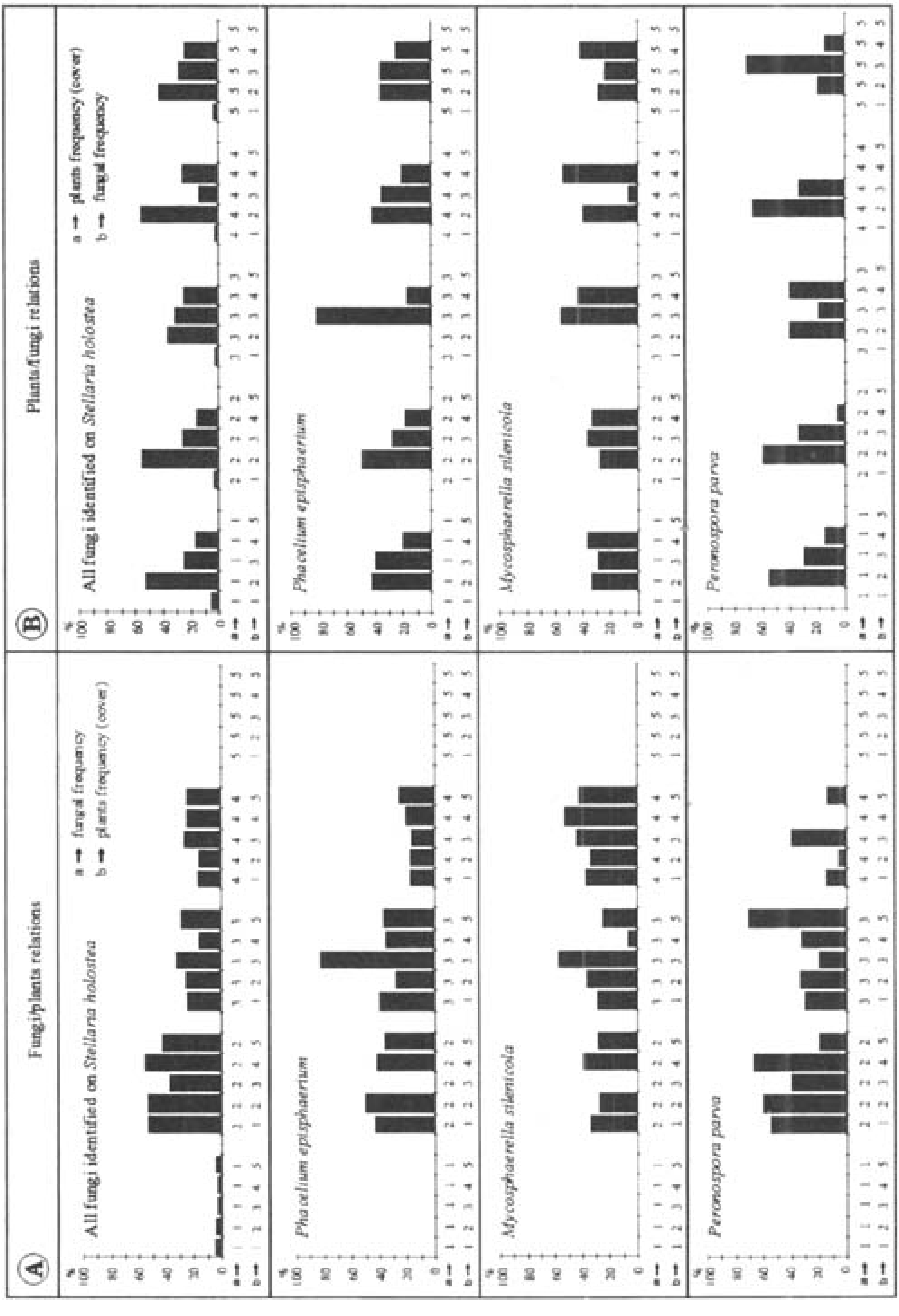

Fig. 4. Comparison of two way analyses of (A) fungi/plants and (B) plants/fungi relations (on the base of total number of records)
(A) 100% = sum of e.g. 1.2, 2.2, 3.2, 4.2, and 5.2 positions;
(B) 100% = sum of e.g. 2.1, 2.2, 2.3, 2.4, and 2.5 positions

of single fungal species this information is less regular, but it is easier to estimate their preferences in relation to a particular density type of host plants.

Both forms of analysis – in dependence on the requirement – can be used jointly or separately. It seems to be not only advisable but quite indispensable to determine the frequency of fungi and their host plants in every case of future studies. This concerns both the studies conducted in permanent plots and in large areas.

Utilization of permanent study plots

The information obtained from studies on parasitic fungi in V-100 (144 ha) and in one hectare of *Tilio-Carpinetum* (plot No 40) is much more exact compared to that from all to date studies in this community. Systematicness of investigations accompanying studies conducted in permanent plots allows observations of the phenology of fungi and seasonal changes in the structure of their distribution (Fig. 5).

The results obtained so far show that studies on permanent plots contribute, among other things, to: (a) carrying out much more accurate studies without degrading the floristic information; (b) gathering new information with regard to quantity and quality; (c) full statistical analysis of the gathered data; (d) following up the association of pathogens with plants (potential hosts) and with the inner, structural differentiation of the studied object (Fig. 3); (e) enabling comparison of the results of studies conducted in different and even very distant regions (Fig. 6).

The future mycological-ecological studies, using permanent study areas seems to be indispensable. The size of areas, their number (statistically significant) and distribution depend on the purpose of studies and the planned minuteness rate.

In studies of a high minuteness rate the best solution is utilization of already existing permanent research areas adjusted to ecological studies. As a rule such areas are ecologically-phytosociologically well described and possess a rich research documentation. Material can be taken in the areas, or selectively in systematic or random sampling (F a l i ń s k i, msc.) (Fig. 7A).

In floristic-ecological studies estimated in larger areas a system of permanent plots with distribution adjusted to various biochor sizes can be used within a definite community (Fig. 7B). Research plots need not be literally permanent. Here, a system of permanent, precisely localized points can be used which allow each time reconstruction of areas determined earlier. The most essential element of such studies is to provide for oneself (and future researchers) repeatability of studies, i.e. return to the same places and taking samples from the same areas. A close co-operation with an ecologist or phytosociologist is recommended when choosing a research area.

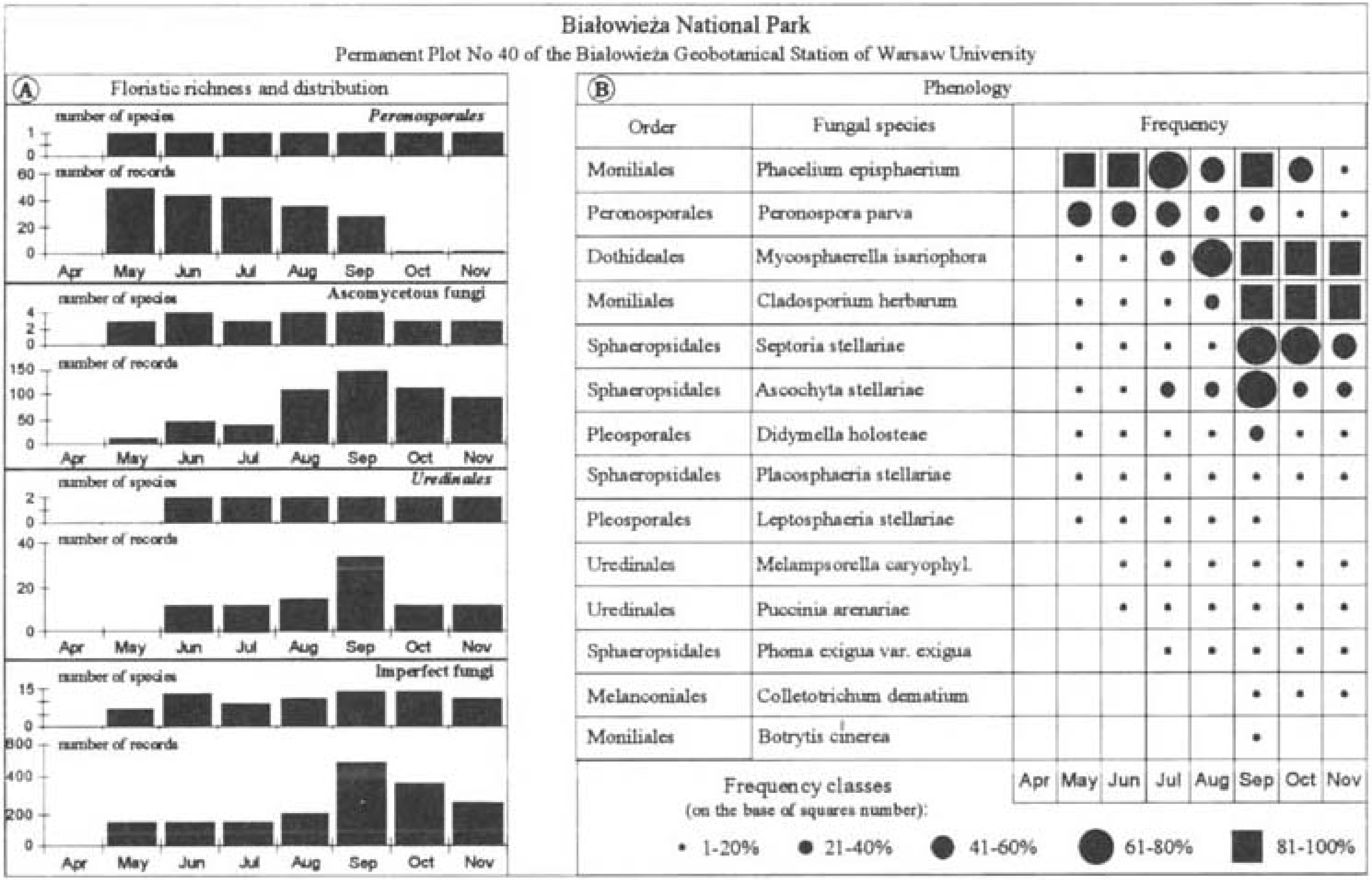

Fig. 5. Floristic richness, distribution and phenology of fungi identified on *Stellaria holostea* in the period of 1992-1995

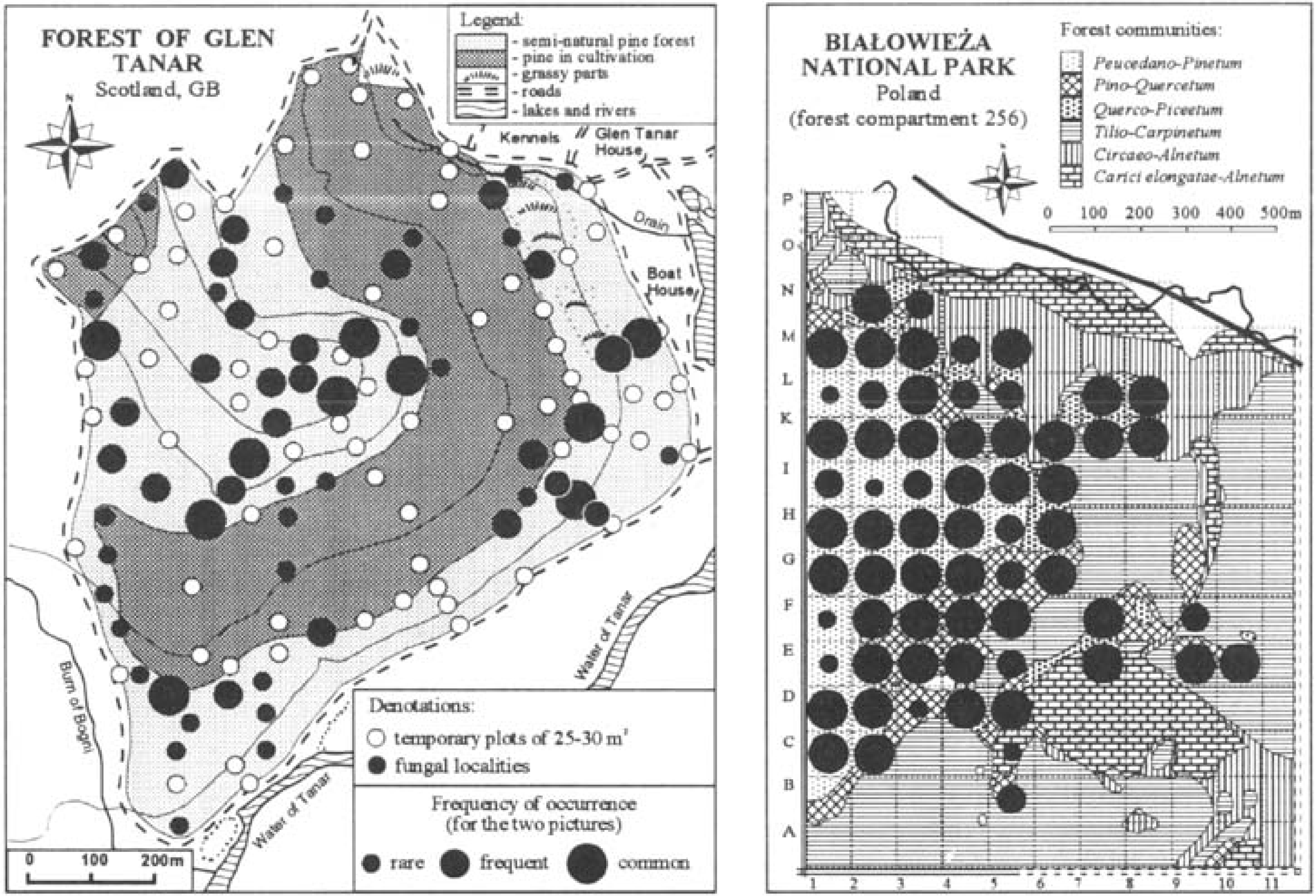

Fig. 6. Occurrence of *Valdensia heterodoxa* (*Moniliales*) on *Vaccinium myrtillus* in coniferous forests
Scotland – Caledonian pine forest (area of 200 ha); Poland – coniferous forest communities (area of 144 ha). Source: Mułenko, Woodward 1996, Fig. 3, supplemented

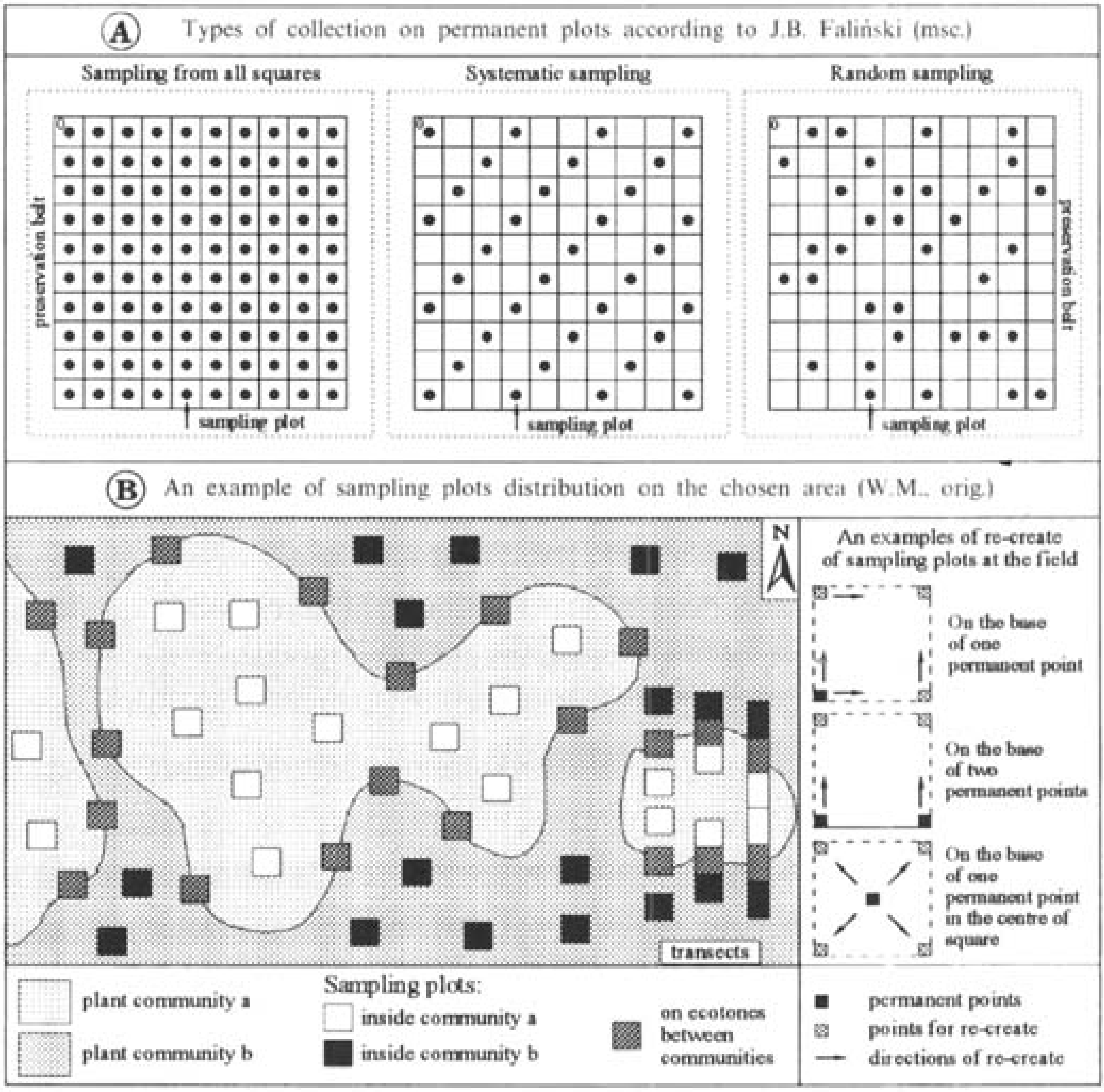

Fig. 7. Examples of permanent plots employ in mycological research at the field

CONCLUSIONS

On the basis of the present state of knowledge of the parasitic mycoflora in natural plant communities it is advisable to:

1) carry out studies in relation to a strictly defined locality and environment-habitat conditions;
2) carry out studies in strictly localized permanent areas of comparable size;
3) limit determination of the frequency of fungi to a 3- or 5-grade scale;
4) estimate constantly (in a range comparable to that of fungi) the frequency of host plants;
5) connect studies on the richness of mycoflora with distribution of fungi;

6) aim at obtaining comparable results, which is indispensable to follow up the occurring changes;
7) adopt methods allowing comparison of results in regard to other groups of fungi.

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Przegląd metod stosowanych w badaniach nad grzybami pasożytniczymi w naturalnych zbiorowiskach roślinnych

Streszczenie

Treścią artykułu jest przegląd stosowanych dotychczas metod badawczych oraz propozycje dotyczące ujednolicenia badań w zakresie sposobu gromadzenia informacji o grzybach fitopatogenicznych w naturalnych zbiorowiskach roślinnych. Przedstawiona dyskusja odnosi się do takich badań, które odzwierciedlają całość flory pasożytniczej w obrębie określonego areału. Nie analizowano powszechnie prowadzonych badań, które dotyczą prostych relacji w układzie 1 gatunek pasożyta — 1 gatunek (lub jednogatunkowa populacja) rośliny żywicielskiej.

Jednym z podstawowych problemów w pracy terenowej jest przyjęcie odpowiedniego sposobu oceny ilościowości (częstotliwości, frekwencji) występowania grzybów. Zakłada się, że sposób opisania występowania grzybów, a więc przyjęta metoda, powinien: (1) odzwierciedlać wzajemne relacje w układzie patogen-żywiciel, (2) umożliwić zastosowanie przyjętej metody w różnych warunkach środowiskowych, (3) umożliwić powtórzenie badań w różnym czasie, dając porównywalne wyniki.

W ocenie frekwencji występowania grzybów w warunkach naturalnych najbardziej optymalne wydaje się wprowadzenie następujących zasad:

1. Stosowanie skali 3-stopniowej na obszarach o wielkości ok. 1 ha lub większych (Fig. 1A). Metoda 3-stopniowa jest najprostsza, ale także najbardziej uniwersalna. W każdych warunkach środowiskowych pozwala na ocenę trzech podstawowych, a zarazem najważniejszych typów frekwencji — minimalnej, średniej oraz maksymalnej.
2. Stosowanie skali 5-stopniowej na powierzchniach badawczych o wielkości od 25 m^2 do $\pm$200-400 m^2 (Fig. 1 B).
3. Dokładne liczenie wszystkich (zdrowych oraz porażonych) osobników na powierzchniach poniżej 25 m^2.

Nieodłącznym elementem analizy zjawiska pasożytnictwa w warunkach naturalnych powinna być ocena frekwencji żywicieli. Na stałych, lecz niezbyt wielkich powierzchniach badawczych optymalne wydaje się zastosowanie skali 5-stopniowej (Fig. 1B) lub odpowiednia modyfikacja skali 10-stopniowej. Podczas badań na dużych areałach najpewniejsza jest skala 3-stopniowa.

Proponowana ocena frekwencji obu organizmów (patogena i jego żywiciela) pozwala na analizę dużej liczby związków, dając dość szczegółowy obraz zależności występujących w naturalnych siedliskach. Przy zastosowaniu skali 3-stopniowej możemy przeanalizować łącznie 9 różnych typów kombinacji. Przy zastosowaniu skali 5-stopniowej liczba ta wzrasta do 25 (Fig. 1). Łączna analiza obu typów frekwencji umożliwia uzyskanie odpowiedzi m.in. na następujące pytania: (a) jakie typy związków w określonym siedlisku występują najczęściej?, (b) jaki typ frekwencji grzybów dominuje w obrębie określonego typu zagęszczenia roślin?, (c) przy jakim zagęszczeniu roślin choroba występuje w największym natężeniu (Fig. 4)?

W badaniach mikologiczno-ekologicznych niezbędne jest stosowanie stałych powierzchni badawczych. Wielkość powierzchni, ich liczba oraz rozmieszczenie zależą od celów badań oraz planowanego stopnia szczegółowości. Powierzchnie badawcze nie muszą być stałe, w dosłownym rozumieniu tego słowa. Można tu użyć systemu stałych, ściśle zlokalizowanych punktów, które umożliwią każdorazowo odtworzenie wyznaczonych wcześniej areałów badawczych (Fig. 7).

Najważniejszym celem badań terenowych jest konieczność zapewnienia sobie (oraz w przyszłości innym badaczom) powtarzalności oraz porównywalności wyników badań. Spełnienie powyższych warunków pozwoli na połączenie badań nad bogactwem mikoflory z rozprzestrzenieniem grzybów. Tym samym możliwe stanie się, także na bazie pasożytniczej mikoflory, śledzenie zachodzących w środowisku zmian.