Fungi on the leaf – a contribution towards a review of phyllosphere microbiology from the mycological perspective

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Abstract: The term phyllosphere is used here as the space covering and including the living plant leaf. Study of the microbiology of this microhabitat is usually focused on bacteria. In this minireview, the diverse ecological roles of fungi with examples of systematic groups are introduced. In the phyllosphere, superficial ("ectophytic"), parasitic ("ectendophytic") and endophytic fungi can be distinguished with respect to their position relative to the leaf surface. Methodological problems of studying fungal diversity of the phyllosphere are the general lack of knowledge about fungi, ignorance of ephemeral fungi which are present as numerous spores falling onto the leaf but without colonizing it, and the inconsistencies of different methods each covering only a part of the fungal diversity with low overlap between the approaches.

1. General characterization of the phyllosphere

The term phyllosphere was introduced in a mycological publication by LAST (1955) in analogy to the term rhizosphere, derived from the Greek "phyllos", leaf, and "sphaira", globe, and in present microbiology refers to the space covering and including the living plant leaf. An alternative, slightly different term is phylloplane, focusing on the outer surfaces of the leaf. Since the leaf surface is not plane when seen with the microscope, but shows a three-dimensional relief, the term phyllosphere is preferred in microbiology. The total size of the phylloplane, however, has been estimated as 640,000,000 km^2, exceeding the total surface area of the Earth of 510,000,000 km^2 (LINDOW & BRANDL 2006). Plant leaves are the main terrestrial source of primary production of carbohydrates and O$_2$. Because of the size and productivity of the phyllosphere, its study is of immediate relevance to terrestrial life on Earth.

The phyllosphere provides nutrients and shelter for a large range of highly diverse organisms, such as insects, spiders, tree frogs and epiphyllous mosses and liverworts, and, of course, a high diversity of microorganisms. Compared to the subterranean rhizosphere with its relatively stable temperature, moisture, nutrient availability and other abiotic environmental conditions, the conditions on the phyllosphere can be considered an extreme habitat, with temperature highs of 50 °C or more, exposure to harmful ultraviolet rays, drastically changing erratic periods of drought and of inundation, and limited access to nutrients (LEVEAU 2009). The limited resources also induce particular adaptions and strong competition between the species that colonize the phyllosphere (SCHREIBER et al. 2004). The plants themselves also developed strategies in order to reduce colonization of their leaves by other organisms. The phytopathological literature provides a vast amount of studies about the chemical strategies of plants that prevent that they are penetrated by alien orga-

nisms. A general physical strategy against superficial colonization is the complex three-dimensional arrangement of the outer periclinal epidermis cell wall and the minute cuticular waxes which are excreted through pores from the underneath epidermis cells. This complex relief is specific for the plant species and even for each of both sides of the typical leaf (Fig. 1). It provides the leaf with water repellence, commonly known as "lotus effect". It was first and most detailed demonstrated in the upper leaf surface of lotus (*Nelumbo nucifera*). Water droplets do not spread flatly on such surfaces but remain globular and by their own weight run easily off from the surface. At the same time, particles crossing their way are absorbed and removed from the leaf by the falling droplet. The particles may also be cells of microorganisms. The beneficial lotus effect against microbial colonization has been proven in a powdery mildew infesting lotus leaves (CERMAN 2007). Additionally, plant species-specific hairs, glands and other surface structures on the leaf provide the phyllosphere with a largely uninvestigated universe of potential interactions with microorganisms.

Fig. 1: Reliefs of abaxial (left) and adaxial (right) side of the same leaf of *Magnolia liliiflora* (modified from: KIRSCHNER 2010).

2. Phyllosphere microorganisms

Organisms that have to survive in this habitat, usually have developed strategies in order to overcome the adverse abiotic and biotic conditions of the phyllosphere. Microbiologists are, therefore, particularly interested in the potential of phyllosphere microorganisms to produce particular compounds in biotechnology. Phyllosphere microorganisms also have a strong economic impact as pathogens of plants or as antagonists of plant parasites. Plants developed strategies in order to avoid (or support) the colonization by microorganisms. Such strategies, like the lotus effect can be applied in bionic and other approaches. From a global ecological view, phyllosphere microorganisms can be considered the first stage decomposers of plant leaves since they start nutrient recycling on the living, senescent or freshly dead leaf.

Microorganisms colonizing the phyllosphere are prokaryotes including cyanobacteria, filamentous non-lichenized and lichenized fungi and yeasts, algae, protozoa, nematodes and rotifers (Serusiaux 1989). Microbiologists in their studies of phyllosphere microorganisms generally select bacteria and disregard all other microorganisms. In their reductionist view, bacteriologists claim bacterial "dominance" in the phyllosphere based by cell numbers (10^2–10^{12} cells/g leaf), but high cell number of very small cells may not be as ecologically significant as fewer numbers of much larger cells. Biomass or further other parameters than cell number are probably more adequate non-biased parameters for comparing the impact between different microbial phyla. The bacterial "dominance" in bacteriological investigations is also biased by a common preference for soft-leaved herbaceous plants for study. Even in bacteriological reviews of phyllosphere microbiology, the role of bacteria is seen in the context of the succession of microorganisms, with bacteria playing the main role on young leaves, yeasts on mature leaves and filamentous fungi on mature to senescent leaves (Whipps et al. 2008).
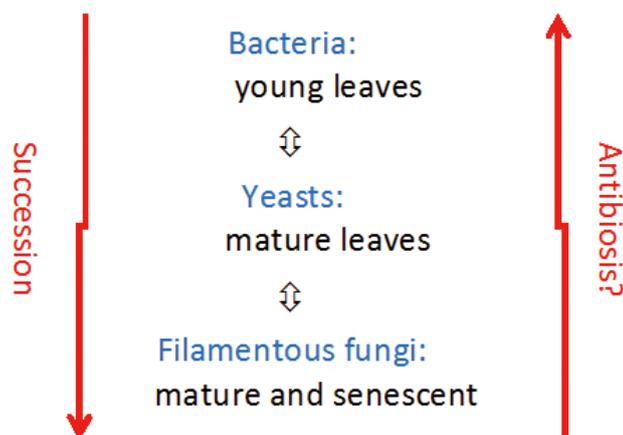

Fig. 2: Diagrammatic representation of microbial succession on the leaf as it is known from phyllosphere microbiology (Whipps et al. 2008).

3. Ephemeral fungal components of the phyllosphere

Although the importance of fungi on leaves is recognized in mycological biodiversity studies, common biological misconceptions lead to inadequate methods and conclusions. Fungi associated with the phylloids of non-vascular plants such as bryophytes are not included here, because in terms of systematics and ecology, fungi differ from those of vascular plant leaves (see Döbbeler 2018 in this volume). It can be estimated that the majority of fungi in and on the leaf originate from fungal propagules (spores and fragments of hyphae or thalli) that have accidentally fallen onto the leaf by dispersal through the air. It is necessary to distinguish between the propagules that establish them-

selves as constant part of the phyllosphere microbiota by continuous growth and at least at a later stage even by reproduction and those propagules that remain dormant or, after a period of germination with hyphae and/or appressoria cease to grow (Fig. 3), and eventually may fall off from the leaf again, or be ingested by arthropods or decomposed by other microorganisms. Such fungi are ephemeral, but may constitute a high proportion of the microbial propagules on healthy leaves, as can be estimated from the spore numbers in the air, since outdoor spore concentrations are usually found at ranges of n ×1000 per m^3 (ELBERT et al. 2007). It can be estimated that only a very small percentage of the spores from the air manage to establish themselves constantly in the leaf, whereas the majority of spores may serve as source of nutrients to other leaf-associated organisms. None of these relations have been investigated systematically or quantified, but again from the study of fungal bioaerosols, we have estimates of 7–49 ng glucose and fructose per m^3 (ELBERT et al. 2007). Since ephemeral fungal propagules do not establish on the living leaf, they should be excluded in mycological biodiversity surveys of leaves. Such studies, however, are usually sophisticated only in technical molecular and statistical analyses, but do not exclude these ephemeral fungi so that the conclusions about fungal diversities associated with the leaves show general overestimations and limited biological significance.

Fig. 3: An example of a presumptive ephemeral fungus in the phyllosphere. Conidium of *Alternaria* sp. with numerous germination hyphae from the adaxial surface of a healthy leaf of *Hibiscus rosa-sinensis*, Taiwan. If the leaf remains healthy, the germination hyphae will not be able to penetrate into the leaf and, after exhaustion of the nutrients stored in the conidium, eventually disappear.

4. Phyllosphere fungi

4.1 Specific phyllosphere fungi

The fungi establishing themselves constantly on or in the leaf have high significance for the phyllosphere. They are attempted here to be classified according to their interaction with the plant tissues as

- Superficial ("ectophytic"): exclusively external growth, no aggressive nutrient uptake

- Plant-parasitic ("ectendophytic"): penetration into the leaf, causing disease symptoms by aggressive nutrient uptake from the plant, spores released outside the leaf

- Endophytic: exclusively internal growth, no disease symptoms

Because the term "endophytic" is well-established, the two other corresponding terms "ectophytic" and "ectendophytic" can be used in order to make the differences more clearly, but because of their rare use are not preferred here.

Superficial and plant-parasitic fungi often form macroscopically conspicuous white, brown, or black layers or spots on the leaf, which also indicates the importance of these fungi compared to the usually invisible bacterial colonies. Because superficial and plant-parasitic fungi are at least partly exposed to the external environment on the leaf surface, they show more conspicuous responses to changing abiotic conditions than endophytic fungi that live in a more stable, sheltered environment. After spore dispersal and contact with the leaf, adhesion and – in non-superficial fungi – penetration are the most crucial steps for establishing growth on the new substrate and depend on an optimal species-specific correlation between temperature and the time of leaf wetness. Such correlations are measured by automatic systems in governmental agricultural stations so that warnings about economically important plant pathogens, like the apple scab fungus *Venturia inequalis* (COOKE) G. WINTER, are announced to farmers during periods with high probability of crop infection by the pathogen. Superficial microorganisms themselves can actively change the external environment of the phyllosphere by increasing the wettability of the leaf surface and by this improving the conditions for their own growth and possibly the growth of secondary microbial colonizers (SCHREIBER et al. 2004).

4.2. Superficial fungi

The fungi that establish firmly on the surface of the leaf without penetration according to their growth form or nutrition source can be classified as superficial foliicolous yeasts, honeydew fungi (sooty molds), filamentous fungi parasitic on other fungi, insects and other organisms on the leaf, foliico-

lous lichens, or growing on air-borne detritus (e.g. pollen), and fungi without known nutrition source.

4.3. Superficial yeasts

For the biology of foliicolous yeasts, the reader may refer to the excellent review by FONSECA & INÁCIO (2006). Among the fungi growing and reproducing in a one-celled stage by budding, basidiomycetous yeasts are dominant in the phyllosphere. Most of them are characterized by forcible discharge of the daughter cells (ballistospores) which are considered an adaption towards enhanced dispersal to the patchy leaf habitat (Fig. 4). Physiologically, they also differ from yeasts of other habitats (e.g. soil), by their oligotrophy and photoprotective pigments (carotenoids, mycosporines), considered as adaption to the limited nutrients and high solar irradiation on the leaf surface. A common feature of most yeasts in all habitats, a slimy extracellular matrix, may on the leaf surface have particular functions for attachment and water balance. In spite of numerous studies of the biodiversity of foliicolous yeasts showing this general picture and increasing new species every year, NAKASE (2000) stated that "the role of … yeasts in the phyllosphere is not clear." This deficiency is based on the routine investigation method of washing the cells from the leaves followed by isolation of pure cultures on conventional media. Only direct observation with "simple" microscopy reveals the interactions with the phyllosphere, such as parasitism on other foliicolous fungi or on mites.

Fig. 4: An example of a ballistosporic yeast from the phyllosphere, *Rhodosporidiobolus odoratus* from leaf of *Nelumbo nucifera*, Taiwan. Scale bar = 10 μm.

4.4. Honeydew fungi (sooty molds)

Most species of this ecological group can be classified in the systematically defined groups Antennulariellaceae, Capnodiaceae and Metacapnodiaceae (Capnodiales, Dothideomycetes, Ascomycota) and some other ascomycetes. They are saprobic fungi on excretions of plant-sucking insects (aphids, scale insects etc.), in some cases on excretions of the plant itself, and form black superficial mycelial layers on the substrate (Fig. 5). Plant-sap sucking insects take up the phloem sap which is rich in sugars but poor in all other nutrients. In order to gain sufficient amounts of the other nutrients, the insects excrete the surplus sugar in form of honeydew. The sooty molds grow exclusively on this sugar solution, without any penetration into the plant. They may only cause some reduction of photosynthesis by reducing the access to light of the underneath leaf tissues. The biology and generic classification of teleomorphs and anamorphs was excellently reviewed by Hughes (1976). A similarly excellent revision of coelomycetous anamorphs of these fungi on the species level has hitherto not been undertaken so that several of the currently used names are doubtful. Major problems are that these fungi on the one hand can be highly pleomorphic, i.e. the same species can have two or three different conidial stages, and on the other the co-occurrence of different species forming together the same black mycelial layer. The lack of specificity to the carrier plants (as illustrated by finding sooty molds on cars parked some days in the vicinity of plants with high honeydew production) also makes species identification difficult.

Fig. 5: A sooty mold (*Chaetasbolisia* sp.) on leaves of *Cephalotaxus* sp. in the Botanical Garden of Frankfurt am Main, Germany.

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4.5. Filamentous fungi parasitic on other fungi, insects and other organisms on the leaves

Insects, mites and fungi associated with leaves are themselves parasitized by a broad range of fungi. A high degree of host specificity of many of these hyperparasitic fungi is responsible for their high diversity. For example, *Ampelomyces quisqualis* Ces. is restricted to powdery mildews, *Sphaerellopsis filum* (Biv.) B. Sutton to rust fungi, and species of *Spiropes* to black mildews and similar dark-pigmented ascomycetes. It is likely that several species of ballistosporic yeasts form teleomorphs that are mycoparasitic on other foliicolous fungi (R. Kirschner, unpublished). Hyperparasites that occur in temperate regions and are easily cultivable have been studied in detail under the promise of potential biological control of the plant pathogenic host fungus, whereas the majority of hyperparasitic species, particularly those restricted to the tropics or being uncultivable are poorly known. Many parasitic fungi are not necessarily restricted to the phyllosphere, but occur wherever the host lives. If the host, however, is mainly limited to leaves, such as larvae of whiteflies (Aleyrodidae), their specific parasitic fungi are also typical phyllosphere organisms, such as species of *Aschersonia*.

4.6. Foliicolous lichens

Lichens are symbiotic associations of fungi and algae/cyanobacteria forming structures, compounds and ecological niches that cannot be formed by a single partner alone (see several contributions of this volume). More than 800 species of lichens are known to live predominantly on leaves (Lücking 2008). These foliicolous lichens are usually widespread in quantity and in species numbers in tropical and subtropical areas with high air moisture, with a low proportion of endemic species, and form small, short-lived thalli with life-cycles of 1–3 years (Pinokiyo et al. 2006). Except for species of *Strigula* and few other genera that penetrate through the plant cuticle, the majority of foliicolous lichens appear not to harm the leaf by nutrient uptake or shading, but can even been grown on artificial substrates and, therefore, may obtain their nutrients from aerosol deposits (Pinokiyo et al. 2006).

4.7. Other fungi with unknown source of nutrients

Filamentous fungi that cannot be classified to one of the above nutrition groups are briefly mentioned here. Like many basidiomycetous ballistosporic yeasts, some of these fungi might be oligotrophic, but this hypothesis has not been tested experimentally. Sources of nutrients might be deposits from the aerosol or leakage from the leaf. Pollen deposit can be a source of nutrients sufficient for the development and sporulation of certain fungi (Olivier 1978).

Mycelia and sporulation structures of these fungi are usually inconspicuous. Many microthyriaceous ascomycetes belong here that form superficial hyphae without haustoria and minute brown, shield-like ascomata or conidiomata which by size and color resemble feces of flies and are, therefore, also called fly-speck fungi. In spite of their wide occurrence and high diversity, microthyriaceous fungi are rarely studied, because morphology of these minute structures is demanding and conventional culturing and DNA approaches hardly applicable. Curiously, these fungi are the best known fossil fungi with earliest convincing records from the Jurassic (TAYLOR et al. 2015). Several foliicolous hyphomycetes also belong to the superficial fungi without known nutrition source, although in many cases, they might actually be plant parasitic, but researchers did not find (or look for) penetration into the leaves. Truly superficial fungi of this group might particularly fit to studies about interactions with bacteria that prefer the same microhabitats. Although not having been investigated systematically, the preferred growth of these fungi appear to be the same as that of the bacteria, namely the concave areas along the margins of the epidermis cells which are less exposed to wind and irradiation and are higher in moisture and accumulation of nutrients (Fig. 6). Such a common microniche has the potential of competition and antibiosis between phyllosphere bacteria and fungi, which deserves more study. A casual investigation of *Periconiella machilicola* R. KIRSCHNER showed this growth preference on the leaf combined with antibacterial properties (KIRSCHNER & CHEN 2010).
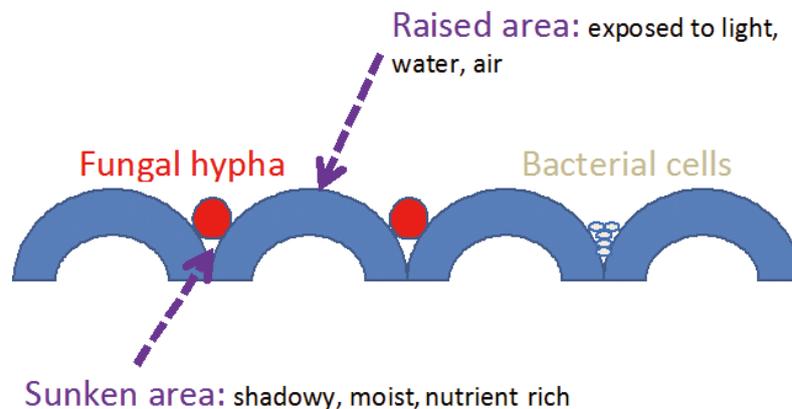

Fig. 6: Diagrammatic representation of microbial competition for the same microniche of the leaf surface of the phyllosphere.

4.8. Plant-parasitic fungi

Plant-parasitic fungi do not only adhere to the plant surface, but also penetrate into the plant, cause disease symptoms during internal growth, and eventually release spores outside. Species of some groups (e.g. tar spot fungi) predominantly grow within the leaf. Only the spores released through ope-

nings in the epidermis and the germination hyphae can be found on the leaf surface. The majority of species of some other groups, however, (e.g. black and powdery mildews) form the main body of mycelium on the leaf surface and penetrate into the plant epidermis cells via haustoria. Many intermediates exist between these two extremes of parasitic fungi with mainly internal or external growth. For specific groups see the contributions of several other participants. Some important groups are listed only:

- Mycosphaerellales/Capnodiales
- Powdery mildews (Erysiphales)
- Black mildews (Meliolales)
- Tar spot fungi (Phyllachorales, Rhytismatales)
- Rust fungi (Pucciniales)
- Downy mildews (Peronosporales)
- Smut fungi (Ustilaginomycotina)

4.9. Endophytic fungi

Endophytes are microorganisms growing exclusively inside living plants without causing externally visible symptoms. Ca. 50 years ago, the term "endophytic" was widely applied to endomycorrhiza fungi on the one hand and the very particular *Epichloë* endophytes of Poaceae. Gradually, study of fungal endophytes was increasing and showed that almost every plant in nature is colonized by endophytic fungi. Whereas the *Epichloë* endophytes early revealed protective properties to the graminaceous hosts against herbivory, the benefits of endophytes in other plants remained obscure for many years. After the breakthrough discovery that the anticancer compound taxol is not only produced by a particular slow-growing *Taxus* tree, but also by its endophyte (STIERLE et al. 1993), the number of studies and publications has been increasing explosively every year. Endophytic fungi can be detected by cultivation-dependent and culture-independent approaches. After collection and surface-sterilization of living plant fragments, internal fungi are allowed to grow out onto cultivation media in the culture-dependent approach, which may include morphological and/or molecular identification of the strains. Or, after total DNA extraction from the plant fragment, fungal DNA is further amplified and sequenced for molecular identification in the culture-independent approach. With respect to the growth in the phyllosphere, three basic strategies of leaf-endophytic fungi can be hypothesized:

Commensalistic-saprob:

- The fungus grows under suboptimal conditions in the living plant, but emerges as primary colonizer as soon as the plant (part) be-

comes dead and by largely avoiding competition from secondary colonizers produces major growth and sporulation.

Latent-parasitic:

- Disease symptoms are not yet observed during the study, but are expected for a later stage of development.

Mutualistic:

- The fungus is provided with shelter, water and nutrients from the plant and enhances the plant's resistance against biotic stress (herbivory, disease) and abiotic stress (heat, salt, drought).

With respect to systematic groups, there is a high ratio of unspecific fungi which occur in almost every study about endophytes, such as species of *Alternaria*, *Fusarium*, *Nigrospora*, Xylariaceae etc. The majority of these species are frequently recorded as saprobes of dead plants, which would indicate their high potential for the commensalistic-saprob role as endophyte. In some cases, they are also recorded as plant pathogens. In a coastal plant, an endophytic fungus is responsible for salt tolerance and survival in coastal habitats (RODRIGUEZ et al. 2008). Other fungi were proven to increase heat tolerance (REDMAN et al. 2002). How such abiotic stress is mediated by endophytic fungi on the molecular level is completely unknown (MÁRQUEZ et al. 2007).

5. Discussion

5.1. Fundamental problems of identifying fungal species

Only 5−10 % of fungal species are estimated to be known to science, with a high proportion of "known" species not or rarely rediscovered after the first description (HAWKSWORTH 2004). The majority of species is only characterized by morphology and substrate, whereas only less than 20 % of the known fungal species are represented with DNA sequences in public databases (HAWKSWORTH 2004). Internal transcribed spacer (ITS) sequences of the ribosomal RNA genes are considered as universal barcode for fungi, but only ca. 15.5 % of the known (< 3 % of the estimated) species have ITS sequences entries (SCHOCH et al. 2012). No real universal DNA barcode, however, exists, but several genes applied in different genera of fungi are more useful for separating species. Up to 20 % of fungal ITS sequences in databases are labeled with wrong species names (NILSSON et al. 2006). The divergent quality of DNA sequences in databases is often not considered by their users, such as the technical quality of sequencing (reading errors, ambiguous positions, simple reads vs. double reads, chimeric sequences); sequences publicly available, but actually unpublished and thus not reproducible (but: many sequences cited as "un-

published" in GenBank actually have been published!); and sequences based on molecular species identifications derived from similar sequences instead of from vouchers identified by other (primary) means than comparisons with DNA data. Although information about described species is easily available by databases (such as Index Fungorum) and voucher specimens can be sent between many countries by post, authors of new taxa often do not compare their newly proposed taxa with all described ones from the same taxonomic rank, but limit their comparison to the few available DNA data and, by this unnecessarily produce new synonyms, which are uncritically taken up by users of DNA databases again.

5.2. Multiple ecological roles of fungi

Although fungi of the phyllosphere are divided here for convenience into superficial, plant-parasitic and endophytic ones, this division is not as clear-cut as it appears in the studies which are by their nature focused on one of these three aspects. Depending on the host plant species, its colonized organ, environment and the particular strain or developmental stage of the fungus, the same fungal species has been recorded as showing superficial, parasitic or mutualistic-endophytic growth. Compared to the numerous reports of fungal species as definite saprobes and/or parasites, proven mutualistic plant-endophyte interactions are still anecdotal. Even in the saprobic and parasitic interactions, the unknown aspects widely outdate the known ones. For example, although many endophytic fungal species have been recorded as saprobes on dead plants, the hypothetical transition from restricted endophytic growth in the living plant to copious growth and sporulation on the dead plant has not been investigated experimentally.

In contrast to the common practice in bacteriology where authors present conclusions about microbial ecology from identification of high taxonomic ranks, fungi belonging to the same systematic group, even the same genus, often have completely different biological roles so that identifications above the species level do not allow conclusions, but only some vague speculations about the biological roles of the fungi in the phyllosphere. A recent example was the discovery of a new species of *Pseudocercospora* in *Ficus microcarpa*. In contrast to the several hundred species of *Pseudocercospora* found to be associated with plant disease symptoms and considered as plant pathogens, the species from *F. microcarpa* forms its conidiophores hidden in the suprastomatal chamber of apparently healthy leaves and could, therefore, be considered "endophytic" (KIRSCHNER 2014). It could be found only by direct observation, whereas cultivation and molecular approaches could only identify "*Pseudocercospora* sp." or a higher taxonomic affiliation. At least some of the saprobic basidiomycetous yeasts of the phyllosphere may have a mycoparasitic hyphal stage which can rather be discovered by direct observation of the phyllosphere

than by the conventional cultivation and genetic approaches. Generally, the species diversity, geographic distribution, substrate specificity, growth conditions, interactions with other organisms and many more fundamental data are unknown in most fungi so that complex ecological conclusions tend to be at least premature.

5.3. Quantification

Bacteriologists have developed sophisticated methods to quantify the cell numbers of phyllosphere bacteria. Comparable methods are largely lacking for fungi. A method for measuring the "coverage" of hyphae on the leaf surface was developed by CERMAN (2007). It is applicable to fungi with predominant external growth on the surface. For quantitative comparison between different groups of phyllosphere microorganisms, biomass is suggested as universal parameter. Hyphae of endophytic fungi have rarely been found by direct observation and then can hardly been assigned to a particular fungal species, which could be done only in monoxenic experiments with a single fungus in a plant. Since immunological approaches proved to be insufficiently specific, quantitative PCR approaches are being developed for plant parasitic fungi. Much effort will be necessary for correlating these new approaches to fungal biomass in the leaf. Even when such methods will be developed successfully, the above mentioned problems will still persist.

5.4. Inconsistency of different methods

Three fundamental methods are presently applied for studying fungal diversity in certain habitats, namely direct observation, cultivation approaches and culture-independent molecular approaches. As common in science, the newest approaches are generally overestimated and the oldest ones underestimated. A combination of all three methods is rarely applied in mycology. A recent example from dune soil fungi was presented by PRENAFETA-BOLDÚ et al. (2014). The authors found that the culture-dependent approach was biased towards easily cultivable species, whereas the DNA-based approach depended largely on the selected primers, and macrofungi revealed by field collection were not detected by the other two approaches. Parallel studies of endophytic fungi with cultivation-dependent and cultivation-independent approaches found that the majority of detected species was different with a small overlap of identical species in both approaches (e.g. GRISAN et al. 2011, GUO et al. 2001, WALKER et al. 2011, ZHOU et al. 2015). In contrary to theoretical expectations, in such parallel studies the culture-independent approach did not increase species numbers nor the accuracy of species identification and did not or only exceptionally reveal non-cultivable species. Highly sophisticated statistical analyses based on data from a single approach, therefore, present

quantitative conclusions only with respect to the applied method, but not to the real diversity of fungi in the given habitat.

6. Conclusions

The view of the phyllosphere as a microhabitat is a strong contribution from microbiology, which does, however not take into account the importance of fungi in this habitat. Collaboration between microbiologists, i.e. bacteriologists, and mycologists will be a breakthrough for a really comprehensive understanding of the phyllosphere ecology. Methods for monitoring and quantifying the hitherto neglected ephemeral fungi and true phyllosphere fungi have to be developed. Fungi have a broad range from exclusively ectophytic occurrence on the leaf, partly internal and partly external growth, to exclusively endophytic life. The concept of phyllosphere including the whole leaf is adopted here without artificially dividing its internal and external compartments. In mycology, the importance of morphological methods is even increasing in the light of accumulating masses of DNA data that cannot be assigned to any known fungal species. The gap can only be filled by patiently searching for morphologically characterizable fungi on and outside the leaf and extracting DNA from such specimens as voucher for hitherto unidentifiable DNA sequences.

7. Acknowledgements

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