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The phyllosphere and the Phylloplane Microbial Biome: A diverse and Dynamic Community

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ABSTRACT
The aerial portions of the plant, primarily the stem and leaves, are known as the phyllosphere, and they are home to a variety of microorganisms. Its distinct niche is based on the spatial richness, diversity, and distribution of microbial communities as well as the impact of biotic and abiotic variables. Plant leaf surfaces, also known as phylloplanes, are a special and difficult microbial biome that is home to a dynamic and varied community of microscopic commensal, parasitic, and mutualistic organisms. These phyllosphere communities, which have particular adaptations and show multipartite connections with host plants and among community members, are shaped by a variety of circumstances. Understanding the fundamental structural principles of native microbial phyllosphere populations will aid in our comprehension of the phyllosphere microbiota and have uses in promoting plant protection and growth. An effort was made to gather prior research in this mini-review in order to have a better understanding of how phylloplane fungus affect the physiology of plants.

KEYWORDS: *Phyllosphere, Phylloplane, Microorganisms, Dynamic, Plant Growth, Protection.*

INTRODUCTION

The entire aerial habitat of plants is referred to as the phyllosphere, whereas the entire leaf surface is referred to as the phylloplane. The phylloplane is a crucial ecosystem from an ecological and economic standpoint because it offers a niche for diverse microbial species. Studies have looked into the phylloplane microbiome as a bioprotectant and growth enhancer for host plants. Phylloplane-microbial interactions and plants lead to higher agricultural crop productivity and fitness. The presence of fungi on the aerial surfaces of plants has long been recognized. Although most emphasis has been placed on the study of fungal pathogens on leaves, stem and fruits, the saprobic fungi on leaves were recorded more than one and a half century ago. Tulasne and Tulasne (1863) described and beautifully illustrated a wide range of fungi including leaf surface inhabitants. de Bary (1866) described *Dematium pullulans* as a fungus commonly occurring on aerial parts of plants. Potter (1910) recognized that surfaces of green plants which under natural conditions appear to be free of microbial life, in fact, support a large population of fungoid/and bacterial germs.

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Despite these early developments, systematic studies of saprobic leaf surface microorganisms began only in 1950s. Last (1955) and Ruinen (1956) independently introduced the term "phyllosphere" to describe the leaf surface habitat. Kerling (1958) suggested that the term "phylloplane" should be used in analogy with the "rhizoplane" for the root surface, thereby stressing the fact that microorganisms actually grow on the leaf surface and not in zones around them. The term "phylloflora" or "epiphytic microflora" or "leaf surface microflora" have also been used. Mahadevan (1975) used a term" phytosphere" possibly to cover all other surfaces of the plant. In the recent decades, interest in this habitat has developed rapidly and a number of reviews and symposia devoted to the subject have been published (Sinha, 1965; Preece and Dickinson, 1971; Sharma and Mukherji, 1973; Dickinson and Preece, 1976; Blakeman, 1981, 1985; Pugh, 1984; Kinkel, 1997; Yang, 2001; Beattie, 2002; Lindow and Brandl, 2003; Whipps *et al.,* 2008; Yan *et al.,* 2022). Although in some cases their role is yet to be established, non-parasitic microorganisms in the phylloplane of living plants have been shown to fix atmospheric nitrogen (Ruinen, 1965; Fokkema, 1976; Skidmore and Dickinson, 1976; Marty, 1983; Shepherd *et al.,* 2005; Cui *et al.,* 2024); to degrade plant waxes and are possibly involved in growth and regulation of the development of plants through introduction of growth regulators (Buckley and Pugh, 1971; Brandl *et al.,* 2001; Mandal *et al.,* 2023). Production of hormones, pigments, volatiles, extracellular polysaccharides (EPS), cross-kingdom signals, and quorum sensing are characteristic facets, which promote proliferation and survival in the harsh and inhospitable habitat of the phyllosphere, exposed to radiation and environmental extremes (Thapa and Prasanna, 2018).

PHYLLOSPHERE MICROBE’S DISTRIBUTION

Phyllosphere microbes can both suppress and stimulate the colonization and infection of tissues by plant pathogen (Lindow and Brandl, 2003). Leben (1965) distinguished two groups of epiphytes on leaf surfaces: (a) residents: which multiply on the surface of healthy plants; and (b) casuals: which are on the plant surface by chance and cannot grow directly on plant surfaces but may grow saprophytically on foreign debris (Fig. 1). The epiphytic microbial distribution is non-uniform in time and space due to various reasons including microclimate, anatomical features such as wax, epidermis, and physiological variations as in the case of leaf leachates (Blakeman, 1971). The pattern of distribution of microbes on leaves is not even- the most common sites of bacterial colonization being epidermal cell wall junctions especially in grooves along the veins (Leben, 1988; Davis and Brlansky, 1991; Mariano and McCarter, 1993) and the stomata (Mew and Vera Cruz, 1986). These are also found under the cuticle (Corpe and Rheem, 1989), in depressions of the cuticle (Mansvelt and Hattingh, 1987) as also near hydathodes (Mew *et al.,* 1984).

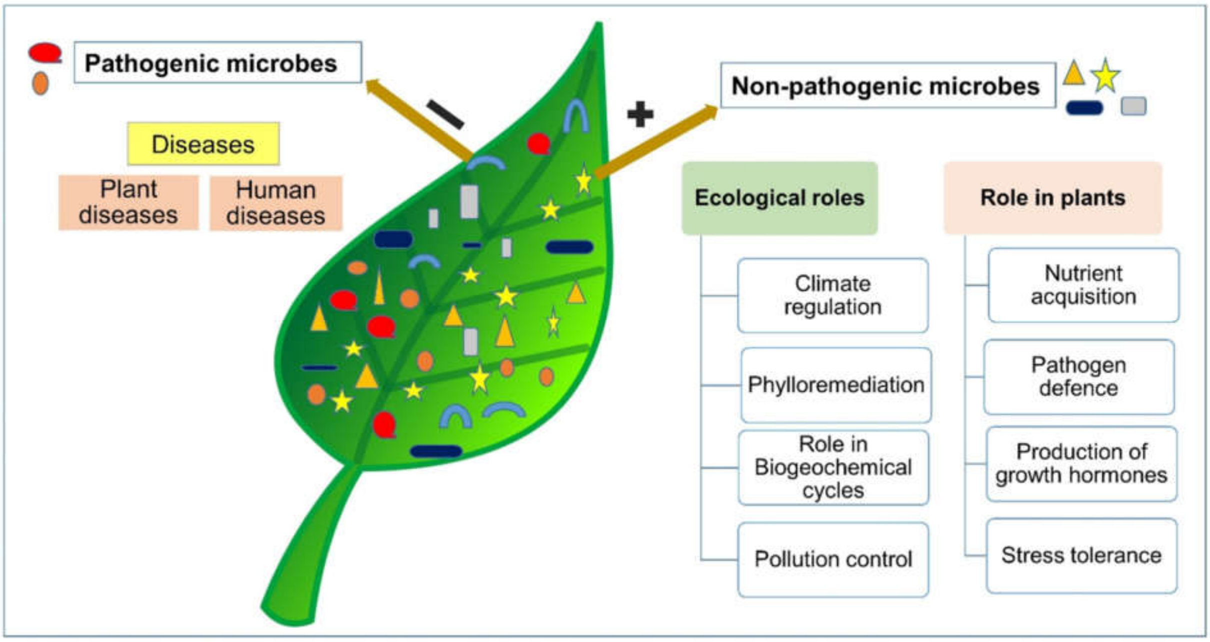

Figure 1: Phyllosphere Microbiome: Functional Importance in Sustainable Agriculture. (Bashir et al., 2022)

Studies on phyllosphere microflora of wheat and barley (Mishra and Tewari, 1978) have revealed variations in fungal populations at different heights of plants and at various stages of leaf development. Seasonal variations in fungal populations were also observed. *Aureobasidium pullulane, Cladosporium* spp. *Botrytis cinerea, Nigrospora sphaerica, Curvularia* spp., *Alternaria* spp, *Fusarium* spp. and *Epicoccum purpurascens* were the common members of the phylloplane. Leaf exudates and extracts had simulatory effect on most of these fungi, and nutrient level of leaf exudates affected the growth of fungi on leaf surfaces.

SUCCESSION OF MICROFLORA

Lindsey and Pugh (1976a, b) studied succession of microfungi on attached leaves of *Hippophae rhamnoides* over a period of three years from before bud burst and until abscission showing that pattern of fungal colonization in general was similar to that described for other angiosperm leaves. *Aureobasidium* was the earliest colonizer continuing until leaf-fall followed by *Sporobolomyces*, *Phoma, Alternaria, Cladosporium*, sterile mycelium, *Epicoccum, Penicillium* spp., *Botrytis* and *Cephalosporium*. For detailed picture of fungal population, the parallel use of a number of techniques in any given study was also suggested.

Phylloplane fungi of *Phaseolus vulgaris* have been studied by Dickinson and O'Donnell (1977) with special reference to behavior of *Cladosporium cladosporioides, Alternaria alternata* and *Sporobolomyces roseus* on attached leaves, under field conditions and in growth rooms. Thompson *et al.* (1993) and Inacio *et al.* (2002) concluded that *Cladosporium* and *Alternaria* are the most abundant fungi on the leaves although several other genera like *Aspergillus, Penicillium, Mucor* and *Acremonium* are also found. El-Kady *et al.* (1995) compared the phylloplane fungi of some herbal plants belonging to Labiatae, Solanaceae and Umbelliferae. Highest number of fungal taxa were recorded from Solanaceae. *Cladosporium, Aspergillus* and *Alternaria* were the most common genera. Kinkel *et al.* (2000) suggested that plant species appear to influence the microbial carrying capacity of a leaf- the total number of culturable bacteria being more on broad-leaf plants such as cucumber as compared to grasses. Yang *et al.* (2001) showed that community structure leaves from individuals of the same species is similar but varies significantly between the species. Jacobs and Sundin (2001) believed that pigmented bacteria dominate leaf surfaces presumably because solar radiations influence the ecology of phyllosphere. Lee and Hyde (2002) recorded greater abundance of fungi on the upper surface of the mangrove leaves than lower surface. Some of the fungi were restricted to lower surface. A distinct seasonal pattern was also observed-fungal abundance increasing in the late summer and being highest in the transition period between summer and winter. Grube *et al.* (2011) studied the black (melanised) fungi and bacterial communities in the phyllosphere of grapevine to examine the suggestion that black fungi shape bacterial biodiversity, they suggested that no such correlation does exist (Fig. 2).

FACTORS AFFECTING COLONIZATION

Humidity was shown to be an important factor in influencing the extent of leaf colonization. Phylloplane microflora of *Larix decidua* has been studied by McBridge and Hayes (1977) with special reference to variations in microbial populations on ageing leaves. Species composition of leaf surface microorganisms was found more or less similar to that reported for other plants. Leaf age rather than weather or air spore inoculum was observed as prime factor in the development of yeasts and bacterial populations on leaves. Hyphal development of filamentous fungi could occur only on old leaves.

During their study on phylloplane fungal flora of *Brassica campestris* and *Brassica napus*, Tsuneda and Skoropad (1978) observed three main groups of fungal populations. The first group which included species of *Alternaria, Aspergillus, Botrytis, Cercospora, Doratomyces*, *Humicola, Mucor, Trichoderma* and *Verticillium* occurred rarely throughout the growing season, whereas the second group represented by species of *Arthrobotrys, Acremonium, Coprinus, Dendriphion, Gilmaniella, Scopulariopsis, Stachybotrys, Stigmina* and *Torula* developed mostly on senescent leaves. The third group included the fungi isolated commonly throughout the growth stages of the plant and was represented by *Alternaria alternata, Cladosporium* spp. (mainly *C. herbarum*) and *Fusarium* spp. (mainly *F. avenaceum*). There was also a group consisting of *Drechslera* spp., *Trichoderma harzianum, Trichothecium roseum* and *Phoma* sp. intermediate between first and third groups.

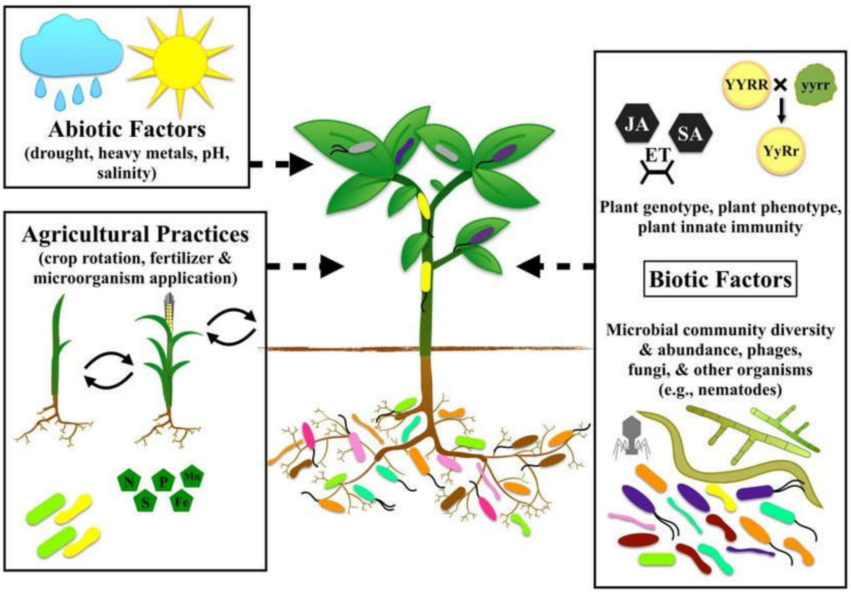

Figure 2: Factors affecting the plant microbiome. The plant's microbiome is affected by many. (Zhang et al., 2021)

Irvine *et al.* (1978) investigated phylloplane fungi of *Acer platanoides* with special reference to effect of inhibitory leaf substances on their seasonal activity and growth. Leaf surface fungi were mostly those reported earlier for other angiosperms in temperate climate. The most common members were species of *Cladosporium, Epicoccum* and some yeasts with small numbers of *Alternaria* spp., *Botrytis* spp., and *Aureobasidium pullulans*. Gallic acid was found as important inhibitor to phylloplane fungi.

Phylloplane microfungi of barley, triticale and eggplant have been studied by Garg *et al.* (1978). It was possible to classify the non-parasitic mycoflora as 'casuals' and 'residents'. Most of the fungi were common to all three plants and, in general, were those reported on other plants in different parts of the world. It was also suggested that use of a host of techniques would be helpful to obtain detailed distribution of leaf surface mycoflora.

Diem (1974) emphasized that leaf washing technique revealed the presence of important populations in the phylloplane and direct observation is complementary. Sharma (1982) reviewed various techniques used for studying fungal succession on leaf surface including direct methods (direct observation, impression films, clearing, scanning microscopy/phase contrast microscopy, fluorescent antibody technique, infra-red microphotography), cultural methods (spore fall, plating, damp chamber) etc., and opined that a combination of balanced direct observational and cultural methods only could provide information on detailed composition of fungal communities, duration periods of fungal taxa and their distributional patterns. Lee and Hyde (2002) compared the utility of light microscopy, scanning electron microscopy and leaf washing methods to study the phylloplane fungi of mangroves; light microscopy was found to be more efficient than SEM. Rasche *et al.* (2006b) demonstrated that the profiling of phyllosphere communities based on culture-dependent methods is likely to be inaccurate and may underestimate diversity. However, culture-independent approaches have not yet been used to characterize fungal diversity in the phyllosphere (Whipps et al., 2008).

The colonization of leaf by microbes is influenced by rainfall and high wind (Kinkel, 1997; Zak, 2002) as also by agricultural practices like harvesting and cultivation (Lacey, 1996; Lighthart, 1997; Lorenzini *et al.*, 2023). Immigration of microbes onto the leaf surface can take place through impaction, sedimentation, rain splash or contamination from soil (Lacey, 1996; Griesser *et al.*, 2024).

CONCLUSION

Many different microorganisms, many of which are crucial to plant growth, find a home in the phytosphere of a plant. It has long been known that fungi can be found on the aerial surfaces of plants. The study of fungal infections on leaves, stems, and fruits has received the most attention, but saprobic fungi on leaves have also been identified. The phylloplane is a crucial ecosystem from an ecological and economic standpoint because it offers a niche for diverse microbial species. Studies have looked into the phylloplane microbiome as a bioprotectant and growth enhancer for host plants. Phylloplane-microbial interactions and plants lead to higher agricultural crop productivity and fitness.

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