Bio-Science Research Bulletin
Volume 41, Number 1
January-June 2025: P.62-69
Review Article

Print version ISSN 0970 0889
Online version ISSN 2320 3161

http://www.bpasjournals.com

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

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ABSTRACT
The phyllosphere, or aerial parts of the plant, mainly the stem and leaves, is home to a wide range of microorganisms. The geographic richness, variety and distribution of microbial communities, along with the influence of biotic and abiotic factors, determine their unique niche. 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. Numerous factors influence these phyllosphere communities, which exhibit various adaptations and multipartite relationships with host plants and among community members. Our understanding of the phyllosphere microbiota will be aided by an understanding of the basic structural principles of natural microbial phyllosphere populations, which can also be used to support plant growth and protection. 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.

Received on 10.01.2025, Revised on 24.03.2025, Accepted on 20.04.2025

How to cite this article: Arora V., Kanwal A., Sharma I., and Raj Singh R. (2025). The phyllosphere and the Phylloplane Microbial Biome: A diverse and Dynamic Community. *Bio-Science Research Bulletin,* 41(1), 62-69.

INTRODUCTION

The term phyllosphere refers to the complete aerial habitat of plants, while phylloplane refers to the entire leaf surface. 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. 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 greatest attention, but saprobic fungi on leaves were identified over a century and a half ago. Tulasne and Tulasne (1863) described and beautifully illustrated a wide range of fungi including leaf surface inhabitants. Dematium pullulans is a fungus that frequently grows on plant aerial portions, according to de Bary (1866). 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.

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). Certain traits, such as the production of hormones, pigments, volatiles, extracellular polysaccharides (EPS), cross-kingdom signals and quorum sensing, facilitate growth and survival in the hostile environment of the phyllosphere, where organisms are subjected to radiation and extreme weather conditions (Thapa and Prasanna, 2018).

PHYLLOSPHERE MICROBE'S DISTRIBUTION

According to Lindow and Brandl (2003), phytosphere microorganisms have the ability to both inhibit and promote plant pathogen colonization and tissue infection. Two categories of epiphytes on leaf surfaces were identified by Leben (1965): (a) residents, which proliferate on the surface of healthy plants, and (b) casuals, which inadvertently develop on plant surfaces but may grow saprophytically on foreign waste (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). Microbes do not follow an even distribution pattern on leaves; the most frequent locations for bacterial colonization are the stomata (Mew and Vera Cruz, 1986) and epidermal cell wall junctions, particularly in vein grooves (Leben, 1988; Davis and Brlansky, 1991; Mariano and McCarter, 1993). Additionally, they are located in cuticle depressions (Mansvelt and Hattingh, 1987), beneath the cuticle (Corpe and Rheem, 1989), and next to hydathodes (Mew *et al.,* 1984).

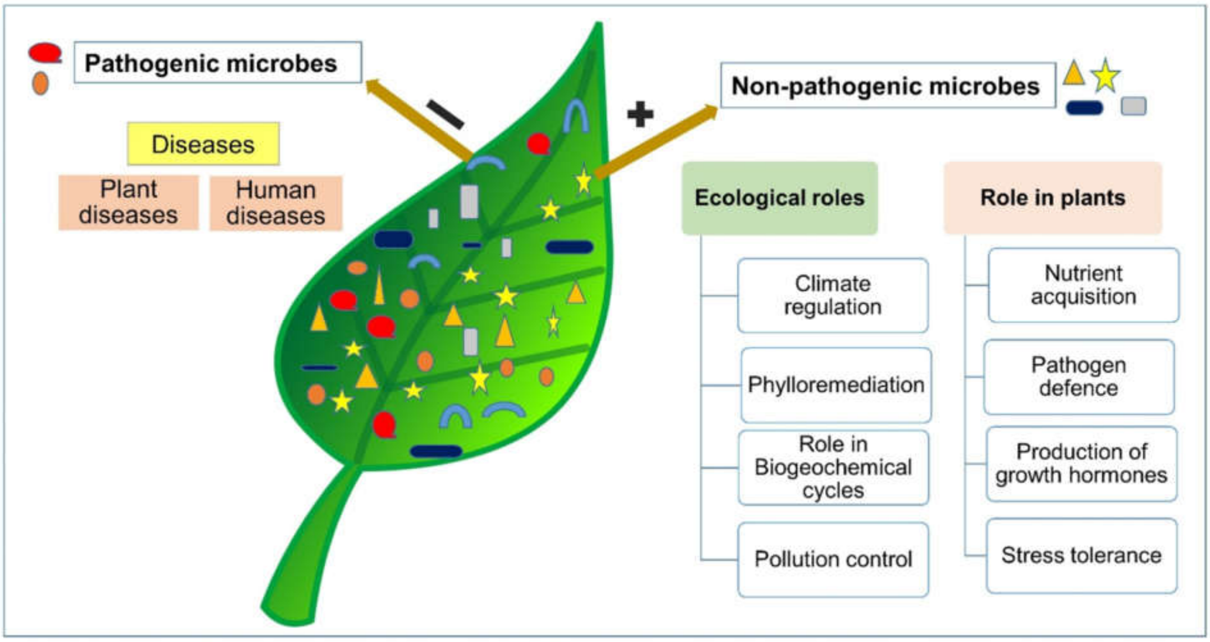

Figure 1: Phyllosphere Microbiome: Functional Importance in Sustainable Agriculture. (Bashir et al., 2022)

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. The majority of these fungi were influenced by leaf extracts and exudates and the growth of fungus on leaf surfaces was influenced by the nutritional content of the exudates.

SUCCESSION OF MICROFLORA

Lindsey and Pugh (1976a, b) examined the succession of microfungi on Hippophae rhamnoides' connected leaves over a three-year period, starting before bud burst and ending with abscission. Their findings demonstrated that the overall pattern of fungal colonisation was comparable to that observed 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*. The parallel application of several approaches in any given study was also recommended for a comprehensive view of the fungus population.

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. The most prevalent fungus on the leaves, according to Thompson *et al.,* (1993) and Inacio *et al.,* (2002), are Cladosporium and Alternaria, while Aspergillus, Penicillium, Mucor and Acremonium are also present. Labiatae, Solanaceae, and Umbelliferae herbal plants were compared for their phylloplane fungus by El-Kady *et al.,* (1995). Highest number of fungal taxa were recorded from Solanaceae. *Cladosporium, Aspergillus* and *Alternaria* were the most common genera. Plant species may affect a leaf's ability to carry microorganisms; according to Kinkel *et al.,* (2000), broad-leaf plants, like cucumbers, have more culturable bacteria overall than grasses. Community structure leaves from members of the same species are similar, but they differ greatly from one species to another, as demonstrated by Yang *et al.*, (2001). Pigmented bacteria, according to Jacobs and Sundin (2001), predominate on leaf surfaces, most likely as a result of solar radiation's impact on the phyllosphere's ecology. 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. The abundance of fungi increased in late summer and peaked during the summer-to-winter transition, indicating a clear seasonal trend. 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 growth of yeasts and bacterial colonies 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.

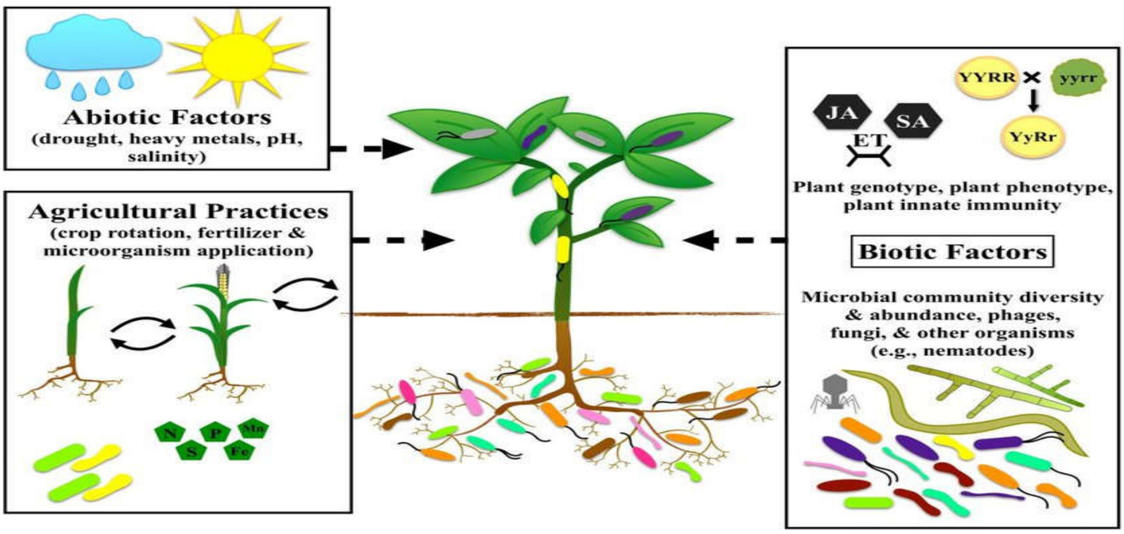

Figure 2: Factors affecting the plant microbiome. The plant's microbiome is affected by many. (Zhang et al., 2021)

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) highlighted the complimentary nature of direct observation and the leaf washing approach, which demonstrated the presence of significant populations in the phylloplane. 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 blend of balanced direct observational and cultural techniques only could provide information on detailed composition of fungal communities, duration periods of fungal taxa and their distributional patterns. To investigate the phylloplane fungus of mangroves, Lee and Hyde (2002) examined the effectiveness of light microscopy, scanning electron microscopy and leaf washing techniques; light microscopy was found to be more efficient than SEM. The profile of phyllosphere communities using culture-dependent approaches is likely to be imprecise and may understate variety as (Rasche *et al.,* 2006b) showed. However, the phyllosphere's fungal diversity has not yet been described using culture-independent methods (Whipps *et al.,* 2008).

In addition to agricultural methods like harvesting and cultivation, rainfall and strong winds also have an impact on the colonization of leaves by microorganisms (Kinkel, 1997; Zak, 2002; Lacey, 1996; Lighthart, 1997; Lorenzini *et al.,* 2023). Microbes may immigrate onto the leaf surface by soil contamination, impaction, sedimentation or splashing rain (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. The presence of fungi on plant aerial surfaces has long been recognized. Most research has focused on fungal diseases of leaves, stems, and fruits, 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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