

1. Introduction :

Fungi on aerial surface of plants :

The presence of fungi on the aerial surface of plants has long been recognised. Although most emphasis has been placed on the study of fungal pathogens on leaves, stems, and fruits, saprophytic fungi were recorded more than a century ago. The brothers Tulasne (52) described, and beautifully illustrated a wide range of fungi, including leaf surface inhabitants. In 1866, de Bary described Dematium pullulans as a fungus commonly occurring on aerial parts. The early description of the sooty moulds (the "soot dew" of Zopf, forming a black, fuliginous coating on living plants) and brown walled cells found on succulent fruits by Pasture, were referred to later by de Bary (1). Meanwhile Pasture (36,37) had recorded the distribution of yeasts on fruits.

Despite these early accounts, interest in saprophytic leaf surface micro-organisms has developed very recently. Last (26) studied the distribution of the members of sporobolomycetaceae on wheat and barley in Britain, Ruinen (45) investigated the occurrence of Beijerinckia a nitrogen fixing bacterium, on leaves in Indonesia. These two workers independently introduced the term "Phyllosphere" to describe the leaf surface habitat. Last (26) suggested that there is a characteristic microflora that may contain many species, and showed that it included yeasts Bullera alba, Sporobolomyces rosens and Tilletiopsis minor.

In 1958 Kerling suggested that the term, "Philloplane" should be used when referring to the leaf surface habitat. This would be consistent with the use of "Rhizoplane" for the root surface. Although the other workers were examining fungi on aerial parts of plants at this time (e.g. Dactylis glomerata (53); Agropyron repens (22); Carex paniculata (40)), they largely directed their investigations to the mycoflora of the dead leaves and inflorescence axes; and as a "neglected milieu". Subsequently interest in this habitat developed rapidly, as may be judged by the major review of the micro-organisms and their activities by Last and Deighton (27) and the publication of symposia devoted to this subject (3,10).

The leaf surface can act as a landing stage for spores and other propagules in the air, which may be deposited by impaction, by boundary layer exchange, by sedimentation under the influence of gravity, and in rain, and splash droplets. It has been described as a spore trap by Gregory (18) who discussed the efficiency of deposition. This will altered by changing the angle of incidence of the leaf to the wind, and also if the leaf surface becomes wet. While wet surface can retain spores better than a dry one, rain falling after spore has been deposited may was it off again. Similarly leaves shaking in wind will set up many eddy currents which might be expected to remove deposited spores.

Once on the leaf surface, the spores derived benefit by diffusion of nutrients from the leaf, and from algae and pollen



grains on the leaf surface. When leaves are infested with aphids, the honey dew forms an abundant source of food. The activities of leaf mining insects might also be expected to cause the release of extra nutrients.

Leaves have been shown to be colonized by fungi from the first unfolding of the bud (42,47). The earliest colonizers are not hindered by competition from micro-organisms. However the potential colonists have to face a number of severely restrictive hazards. Although transpiration may mitigate against extreme desiccation, considerable fluctuations in the relative humidity on the surface are to be expected both diurnally and seasonally. At the same time the leaf surface is not insulated against temperature fluctuations. Most importantly, organisms on the leaf are exposed to the ultra-violet (u/v) radiations in daylight. Thus the phylloplane micro-organisms have to be able to survive transport to the leaf surface, to become lodged there, and then to germinate and grow in this hostile environment (42).

2. Methods of study :

The occurrence of fungi and other organisms on the surface of living leaves of wide variety of plants has been studied in detail, and the fungi have been located and isolated by the use of an increasing number of techniques (9). Lindsey (29) analysed the methods to study fungal succession and found between 1955 and 1973 that the number of techniques used in individual studies rose, he

showed the importance of the parallel use of isolation techniques with methods which enabled direct observation of the surface of the freshly collected leaves.

The use of cultural methods can give only direct evidence of activity of the organism. Counts of bacteria and yeasts may represent the viable cells present on the leaf which were able to grow in conditions provided. However Beech and Davenport (2) pointed out that yeasts can occur as a single cell, as colonies and as short or long pieces of pseudomycelium. Counts of filamentous fungi are virtually meaningless unless they are related to direct observations of length of hyphae; or some other parameters. The use of counts can sometimes be justified (17) to study the fungal potential present before and after a specific treatment the application of fungicide spray, for example. Such counts have relative values only, for the filamentous fungi, they should not be compared with the actual counts which can be obtained for unicellular organisms.

A number of cultural techniques have been used for growing fungi present on leaves, or leaf portions, have been placed in moist chambers, such as that designed by Keyworth (24) to encourage the growth and sporulation of those fungi which were present when the leaves were collected. Leaves or more frequently the discs cut from them, have also been placed on agar media for the same purpose. In both methods spores or other inactive propagules may be stimulated to germinate by the favourable conditions. To overcome this objection

the leaves can be washed to remove loosed lodged spores, leaving any hyphae which have grown on leaf surface. If desired the washing water can be plated out to see what has been removed, and if serial washing (20) is carried out, aliquots of each volume of water can be examined separately. In this way an end point can be found, after which no, or very few, further propagules occur, and only hyphae are presumed to remain. A more drastic treatment is to surface sterilise the leaves, to kill all organisms present on the surface. The sterilant use have included calcium hypochloride, mercuric chloride and silver nitrate. The first two are normally removed by washing after a suitable period of immersion while the silver nitrate can be precipitated by addition of saturated solution of sodium chloride. If the method is carried out effectively, any fungi isolated from such a leaf or leaf disc must have come from the internal tissues, and by implication must have grown effectively on the leaf to be able to get there. An element of doubt may arise when the leaf is infested with aphids or other surface penetrating animals, as the fungus may then have been carried into the leaf in some way, or it may have been stimulated by the released exudates around the puncture.

Two modifications of the plating of leaf portions can be seen in the maceration techniques, and in the use of enzymes to disintegrate the leaf. Maceration is usually carried out mechanically in a blender. If the leaf has not been surface sterilized any organism previously present may be able to grow, or may be inhibited

by compounds released from within the cells. This method must therefore be viewed with suspicion until variety of preliminary tests have been carried out with the leaves being investigated. The use of enzyme (pectinase and cellulase) is best carried out on leaf discs. These are soaked in enzymes, preferably under vacuum to ensure rapid penetration. With relatively little practice, the individual leaf parts can be selected (cuticle or vascular tissues) and incubated separately.

The spore fall technique is an extremely simple and effective way of study, the occurrence of ballistosporic yeasts such as Sporobolomyces on leaf surfaces. The leaf is attached to the lid of a petridish, and the spores are discharged and grown on agar beneath, forming a mirror image of their distribution on the leaf. Both the leaf surfaces should be examined in this way. Surprisingly different results may be obtained if a comparable leaf is lightly pressed on to the agar surface. Buckley (4) obtained S.roseus along the veins of Syca more leaves by the spore fall method, but the impression method yielded mainly A.pullulans. He then used the balloon method in which a balloon is pressed on to the leaf. It is then inflated and pressed on to the surface of an agar medium. In this way the colonies of Aureobasidiuns and Sporobolomyces were separated and grew. There need be no antagonism between them; their different isolation frequencies are merely an artefact of the method used.



Unlike the soil, where direct observation of fungi in situ presents various technical problems; the fungi present on the leaf surfaces can be seen without difficulty. Some thin leaves and colourless parts of variegated leaves, can be mounted on microscope slides and viewed directly. Usually, however it is necessary to clear the leaf first.

With some leaves it is easy to strip off the epidermis, when the surface flora can be clearly seen. It is even easier to obtain an impression of the surface by painting or spraying the leaf with cellulose acetate. Once this has hardened it can be removed and mounted on a microscope slide. The film will be a cast of the surface features of the leaf, and will contain any removable particles which were present.

The scanning electron microscope (SEM) has been increasingly used in the study of organisms on the surfaces of plants. With leaves, the detailed microfeatures such as the structures of hairs and the surface waxes can be clearly seen. At suitable magnifications spores and hyphae can readily be located, and even the erosion of the surface waxes around cells of Sporobolomyces has been seen (30). Similarly the association of fungi with the leaf veins, and the clustering of yeasts and the germination of spores around pollen grains have been observed.

There is great scope for the complementary use of indirect and direct methods for studying fungi on leaf surface. These need to be correlated with laboratory studies of the behaviour of the

fungi, particularly under various stress situations. These could include competition with other members of surface microflora; at diff. R.H. and temp. combinations; when exposed to radiations. A study of their physiology and biochemistry under such conditions will help explain their competitive ability on the leaf surface.

3. Actual Microflora :

The persual of Table 1 reveals that Jowar leaves harbour a rich crop of fungi (62 species). Alternaria triticina, Aspergillus (Table I) Sp. Fusarium Sp. and Curvularia sp. were found most dominant in Rabi season while in Khirif crop Epicoccum nigrum, Colletotrichum graminicola cladosporium sp. and Aspergillus sp. were most abundant.

During both seasons of 1984 and 1985 phyllosphere microflora of four varieties of Jowar viz. SPV 245; SPV 341; SPV 351 and Vasant 1 was studied at Botany Department, Shivaji University, Kolhapur.

It is obvious from the results that with the advance in rabi season from December to March (moderately cold humid) the total population of micro-organisms increased. This trend was exhibited by all the 4 varieties. The pertaining data also indicated that Vasant 1 variety of Jowar showed the highest population of microbes as compared to other three varieties. The total phyllosphere microflora of all 4 varieties increased with fall in temperature and increase in relative humidity. Irrespective of age, variety and season Cladosporium sorghi showed highest population; next in order being Drechslera specifera and third was Aspergillus flavus.

The present investigation on epiphytic phyllosphere microflora of four varieties of Jowar indicated that many factors like maturity of leaf, variety, age of the host plant, and climatic conditions affected the microflora. The population of phyllosphere micro-organisms increased with the advance in age and maturity of leaves as reported by many workers (Last, 1955 on wheat, Kerling, 1958 on Beta vulgaris Ruinen 1956, Hudson 1962 on Saccharum officinarum, Holloman 1967 on Potato). The results of the present investigation on the four varieties of Jowar indicated the a similar trend.

4. Characteristics of Phylloplane Fungi :

In an environment which is conducive to good growth there is wide scope for the survival, development and general encouragement of any variants or mutants within the natural population. In a hostile environment, any deviation from the form which had evolved to survive there might be expected to compete with the parental form and to take over its ecological niche. Such a process may well have occurred on the leaf surface, as there is only a relatively limited group of organisms which regularly occur there and may be regarded as phylloplane inhabitant.

I. Morphological Characteristics :

A number of phylloplane inhabitants possess morphological characteristic which appear to aid their survival. These features are generally similar to those which Nicot (34) found in the fungi

which she isolated from sands of the Sahara region of North Africa. She pointed out that the majority of her isolates were pigmented members of the Phragma and dictyo spored Demataceae, members of the Sphaeropsidales, and dark sterile hyphae. She listed as protective mechanisms against strong light and desiccation the brown pigmentation of mycelium, spores, pycnidia and perithecia generally thick membranes, a high rate of production of generally multicellular spores and a tendency for chlamydospores to be present in groups. Pugh and Williams (43) pointed out that the majority of these characteristics are also possessed by fungi isolated from the aerial parts of Salsola Kali. They added to the list the possible benefit to Epicoccum of having its spores in sporodochia, where there would be some mutual protection. They also discussed the beneficial effects of carotenoid pigments, which may be associated with increased tolerance to u/v radiation, or with enhanced energy reception. Codner and Platt (7) showed that Cephalosporium spp. produced carotenoids in response to light, and Leach (28) found that sporulation in a number of fungi is induced by u/v absorbing substances. There is thus a positive advantage for those fungi which possess carotenoid pigments. The deposition of brown pigment in the wall of fungi with dark hyphae and spores would have a protective effect by acting as light screen. While these pigments are often referred to as melanin they may not fit within the restricted usage discussed by Thomson (51), although melanin has been shown by Bull (6) to be deposited in old hyphal

wall of Aspergillus nidulans, and melanin like substances were found to account for 8.5% of the wall weight of Rhizoctonia solani by Potgeiter and Alexander (38).

Pugh and Williams suggested that fungi with protection against, tolerance of, or an ability to utilise light energy would be useful in aerial environment. These features are certainly to be found in many members of phylloplane mycoflora; although they may not always be obvious particularly when a fungus is grown in pure culture in the laboratory. This qualification particularly applies to Aureobasidium pullulans. In culture it has hyaline hyphae from which hyaline conidia are budded, it is not found in this form on the leaf surface. There it may be present initially as hyaline, thick walled cells, which become pigmented and form chains of dark chlamydospores, and later, dark microsclerotia. It may also occur within the vascular tissues (42).

The other common phylloplane fungi in which these morphological features are not apparent are the yeasts. These may grow in clumps on the leaf surface and thus provide some mutual protection; or they may occur in some naturally protected regions, such as in groove, or in the shelter of leaf hair or other projections. Equally they may have some other form of protection, as di Menna (32) found that the nonpigmented yeasts accounted for 22.5% of the total air spora in New Zealand.

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II. Physiological Characteristics :

A) Nutrition :

While all of phylloplane fungi are able to grow on a wide range of media, some of them possess enzyme systems which are peculiarly adapted to their habitat. Pugh et al., (44) drew attention to the ability of the common filamentous fungi to decompose cellulose although A.pullulus does not possess this ability. They went on to review the enzyme systems reported for these fungi and showed that cellulase produced by Alternaria alternata (A.tenues), Botrytis cinera, Cladosporium herbarum and Epicoccum purpurascence (= E.nigrum); Pectinase has been found with A.pullulans, B.cinera, C.herbarum; A.alternata possesses protease, while B.cinera has cutinase and enzymes for the utilization of fructose, glucose, mannose and glycerol. Amongst phylloplane yeasts Sporobolomyces roseus has been shown by McBride (30) to be able to alter the surface waxes of larch leaves and perhaps to use them as source of energy.

In other studies (26) Sporobolomyces has been reported not to increase in frequency in the vicinity of powdery mildew pustules on cereal leaves, although other yeasts Sporidiobolus iohansonii and Itersonilia perplexaus have been to occur in large numbers around rust pustules. This is a good indication that these yeasts are able to utilise nutrients which exude from leaves, and that they can take advantage of the increased leakage which occurs when the epidermis becomes ruptured, in this case by the rust sori.

B) Radiations :

Light at high intensities generally retards mycelial growth, although there are some reports of stimulation. Spore germination in most cases is increased by light, although germ tubes of B.cinerea are negatively phototrophic. Some hyphae are positively phototrophic, may show zonation effect of light have been briefly reviewed by Page (35).

White light, normal intensities, may not be harmful to the phylloplane fungi. The u/v portion of the spectrum, however, plays a much more important part, and in the laboratory, exposure of fungi to wavelengths around 2600 A° can be lethal. Among the filamentous fungi, those with melanin in their walls are more resistant to u/v exposure than are hyaline species and this resistance increases with greater amounts of pigmentation. It is particularly interesting that exposure to light has been found to induce melanisation in Aureobasidium and Cladosporium. Sussaman (50) has discussed this aspect of fungal survival.

In their study of the effect of u/v on phylloplane fungi Pugh and Buckely (42) found that the multicelled, dark spores of Alternaria and Epicoccum survived in large numbers exposure for 35 minutes, and some spores of Cladosporium also survived; although the majority had been killed after 10 minutes. Hyaline spores of Aureobasidium and Sporobolomyces were mostly killed by exposure for only 5 minutes; although the dark chlamydospores and microsclerotia

of Aureobasidium survived as well as did the multicellular spores of Alternaria and Epicocceum. Some spores taken from colonies of Sporobolomyces after 35 minutes exposure found to be alive; indicating that there was some degree of protection afforded to the inner cells of clump by the outer cells. In the same way with the microsclerotia of Aureobasidium and the multicellular spores of Alternaria and Epicoccum all cells derive benefit from their pigmented walls, while some cells will always also be shielded by others.

Sussman (50) pointed out that the members of Dimatiaceae were the most resistant group of fungi recovered after exposure to gamma radiation from a ^{60}Co source and that there was a direct relationship between the resistance of some fungi and their rate of growth in pure culture when subjected to continuous irradiation at a sublethal level. This ability may not normally be important, but it should be remembered when irradiation is used to sterilise the material, and it also would suggest the fungi which are most likely to be found in areas subjected to heavy irradiation, whether this is caused by explosive devices or during the course, for example, of irradiation for plant breeding experiments.

There must be other forms of protection against radiations available to fungi which occur above the ground. The yeasts which were isolated from the air in New Zealand by di Menna (31) included Cryptococcus diffluence, Debaryomyces hansenii and Sporobolomyces salmonicolor. They were all more resistant to desiccation and u/v



irradiation than were other yeast which were not generally air borne including Candida albicans, C.krusei, Saccharomyces cerevisiae and Torulopsis colliculosa. There are various DNA repair mechanisms which may enable those fungi which are lacking in melanin or other pigments to survive exposure to u/v radiation (19). Sussman (50) asked how significant are the effects of u/v and other radiations in nature. The preponderance of fungi able to tolerate or even utilise these radiations of leaf surfaces and the relative absence of species known to be intolerant at least give an indirect but positive answer.

C) Relative Humidity :

On the leaf surface there will be high relative humidities (R.H.) or actual water during rain and when dew is forming. In dry weather however, the humidity level may approach the ambient, apart from the effect of diffusion shells around the stomata. In windy conditions the effect even of these on the micro environment will be reduced. Consequently, the ability to survive and grow at low level of relative humidity will confer an advantage on the phylloplane organisms. Webster and Dix (54) in their examination of fungi on decaying culms of Dactylis glomerata, distinguished between the primary colonizers Alternaria alternata (= A.teniris), Cladosporium herbarum, Epicoccum purpurascens, Leptosphaeria microscopica and Pleaspora vagans and the secondary colonizers Tetraploa aristata and Torula herbarum. They found that the primary group when

compared with secondary, had a shorter latent period for germination of their spores, a faster growth rate of the germ tubes and a generally faster mycelial growth rate when incubated at 100% R.H. However, there was little difference in the abilities of the mycelia of fungi in these two categories to grow at low R.H. All the fungi tested were able to grow at 95% R.H. and none could grow below 93% R.H. They attributed the success of the primary group in an environment of fluctuating humidities to their abilities to exploit the substrate even in less than ideal conditions, and by their rapid growth to flare up and take immediate advantages of any favourable change in humidity conditions.

More recently, Diem (13) examined the influence of R.H. on the survival and germination of Colletotrichum graminicola, a leaf parasite with hyaline unicellular spores and Alternaria alternata (= A. tennis) with multicellular and Cladosporium herbarum with unicellular melanised spores. Spores of Colletotrichum were unable to survive four hours at 73% R.H. while two dark walled species were able to withstand even desiccation in the presence of Calcium chloride. However, both species showed minimal survival in the middle range of humidities from 53 to 73% and when the time of exposure was increased from 4 to 8 hours, particularly in this middle range. Diem (13) studied Cladosporium on the surface of living leaves which had been exposed to a wet period following a dry week. He found that it was present as short, some what

cylindrical, dark portions of hyphae, where there were alternating living and dead empty regions. He thought that C. herbarum survived in this way on living leaves, becoming active whenever there was a period of suitable humidity.

D) Temperature :

The phylloplane fungi in general are mesophillic, growing well at temperatures of about 20 - 25°C. Pugh et al., (44) suggested that incubation temp. as near the ambient as possible should be used when isolating phylloplane fungi, as incubation at normal laboratory temp. may bias the isolation of the mycoflora. They drew attention to the ability of the common filamentous fungi to grow at temperature around or below the freezing point of water. The lowest temp. mentioned were : Alternaria alternata (as A. tennis) 2°C; Aureobasidium pullulans 1°C; Botrytis cinera 2°C; Cladosporium herbarum 6°C; Epicoccum purpurascens (= E. nigrum) 3°C. While there are no data on the behaviour of these fungi in fluctuating temperatures; their survival on leaves provides indirect evidence about this aspect. Their ability to grow at low temp. will enable them to make use of the generally higher humidities present on the leaves at night.

5. Competition on leaves and use of fungicides :

Various interactions saprophytic phylloplane fungi and leaf pathogenic species have been discussed by several workers

including Van den Heuvel (21), Fokkema (16), Skidmore (49), and Sharma and Heather (48).

An alternative to such approaches to biological control of leaf pathogens is in the use of fungicides. These affect both the saprophytic and the pathogenic mycoflora. Dickinson & Walpole (12) found that the late application of fungicides helped to delay leaf senescence, and this, in turn, resulted in an increase in yield; which was greater than could have been expected solely through the control of leaf diseases. Dickinson and Wallace (11), Kuthubudheen and Pugh (25) found that fungicides such as Tridemorph, Captan and Dicloran respectively had a smaller effect on the population and the activity of phylloplane fungi than did fungicides such as Zineb, Vhiran, Verdasan etc.

6. Other organisms present on the leaves :

In addition to the fungi, many other organisms may be present on and within living leaves. The presence of pollen grains has been shown to have a strong influence on the development of both leaf pathogenic fungi and of phylloplane Saprophytes (8,16). The bacterial flora on leaves contains a greater proportion of pigmented forms, and relatively fewer spore formers than are present in the soil. They also show some nutritional differences, such as greater ability to utilize glucose. Other organisms commonly found on leaves include various lichens and algae, particularly members of Chlorophyceae and the Cyanophyceae (17).

Ruinen showed that the organisms present on the leaf surface may form a layer 22 m/u thick. Interactions between these different organisms and between the organisms and the green plant require much further study. For example, nitrogen fixing bacteria have been reported from both tropical and temperate regions (Beijerinckia sp. and Pseudomonas trifolii respectively). Ruinen (46) suggested that "Vegetation might profit by foliar uptake of the nitrogen bound by the microbial population of phyllosphere in return for nutrient excreted by the leaves". Similarly, Diem (14) and Buckley and Pugh (5) thought that the ability of various members of the phylloplane microflora to produce auxins in vitro could have an effect on plant growth.

An area which is awaiting development is in the interaction between both saprophytic and pathogenic fungi with members of leaf fauna. It has been suggested by Pugh and Buckley (42) that Aureobasidium pullulaus may enter Sycamore leaves via aphid punctures. Can such a route be exploited by other fungi and by pathogenic species? Similarly, are there any differences in the fungal population on leaves which have been infected with leaf mining insects? Can fungal infection be spread by members of the leaf micro-fauna? There is a great potential for further study in such relationships.

7. Conclusions & Fate :

Towards the Future :

There is no doubt that more work is needed on phylloplane fungi. Their role vis-a-via leaf pathogenic species needs further elucidation; their associations with members of leaf microflora have still to be fully explored; and the methods of survival under u/v radiations particularly both saprophytic and pathogenic hyaline species, need to be fully elucidated.

While almost any species can be isolated from leaf surfaces, relatively few of them are true phylloplane inhabitants :- the majority will be mere Casuals, deposited from the ubiquitous air mycoflora. Care must always be taken to separate these transient species. Fortunately this is a relatively easy process using the leaf washing technique.

Are the phylloplane fungi survivors or escapers ? It has been suggested (41) that they escape competition in the harsh environment of the leaf surface and that the available evidence indicates that they cannot compete with the soil fungi when the leaf fall on the ground. Although they can be isolated from decomposed leaves in the litter layer, there is no evidence that they actually grow there. A number of leaf pathogenic fungi have their active growth on the green leaf, followed by the maturation of their reproductive structures after leaf fall (e.g. Rhytisma acerinum, Venturia inaequalis), with several saprophytes (e.g.

Aureobasidium pullulans; Cladosporium sp.), there is a production of chlamydospores and microsclerotia on the leaf when the competitors arrive. Are these types of behaviour analogous ? An exploration of the competitive abilities of these fungi could be rewarding, when the role of secondary sugar fungi growing in association with cellulolytic species could also be assessed.