ANTAGONISM OF MICRO-ORGANISMS FROM JUTE PHYLLOSPHERE TOWARDS COLLETOTRICHUM CORCHORI

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Twenty-six fungi and twelve bacteria were isolated from the phyllosphere of jute. In paired cultures Aspergillus nidulans and Penicillium oxalicum and two strains of Bacillus megaterium were found to be highly antagonistic to Colletotrichum corchori. An ethyl acetate soluble, partially thermolabile antifungal substance was detected in the culture filtrate of B. megaterium (B-23) which significantly reduced spore germination and germ tube growth of C. corchori. More than 30% reduction in germ tube length was recorded at 0.25 dilution of the ethyl acetate extract. Foliar sprays of bacterial suspension and culture filtrates 24 h prior to inoculation markedly reduced the production and spread of lesions on jute leaves by C. corchori.

The phyllosphere is usually inhabited by a variety of saprophytic and parasitic microorganisms which interact among themselves and also with the living host plant. Studies on the phyllosphere have immense potentiality in opening up new lines of research for controlling pathogens, especially those causing foliar diseases, by biological methods (Baker & Cook, 1974; Preece & Dickinson, 1971; Dickinson & Preece, 1976). Anthracnose is an important foliar disease of jute, Corchorus capsularis L., caused by Colletotrichum corchori Pavgi & Singh. In this study, jute phyllosphere has been screened for the presence of organisms antagonistic to C. corchori for possible use in the biological control of anthracnose disease, a disease which cannot be significantly reduced by ordinary chemical means.

MATERIALS AND METHODS

Isolation of micro-organisms from jute phyllosphere

Microbes of jute leaves were isolated following the method of Dickinson (1971). About fifty leaves were washed in 100 cm³ of sterile distilled water on a mechanical shaker for 15 min and the washings centrifuged at 15,000 r.p.m. for 10 min at 4 °C. The resulting pellet was resuspended in 10 cm³ of sterile distilled water and ten fold dilutions made in a range of 10⁻¹ to 10⁻⁶. One cm³ of each of these solutions was incubated in 20 cm³ of either potato-dextrose-agar (PDA) or nutrient agar (NA) (peptone 0.5%, beef extract 0.3%, agar 2.5%, Tuite, 1969) medium in sterile Petri dishes at 30 °C. The fungal and bacterial colonies arising on the agar plates were transferred to PDA or NA agar slants.

Interactions between micro-organisms

Fungi and bacteria isolated from jute leaves were paired separately with C. corchori on PDA medium (pH 5.6) to study colony interactions. Methods outlined by Skidmore & Dickinson (1976) were followed for this purpose. Agar blocks (3 mm diam) containing 10 day-old mycelia of the two fungi were placed 3.5 cm apart on the medium in a Petri dish and incubated at 30 °C for 21 days. Bacteria (24 h old) were streaked on to the medium in Petri dishes about 4 cm away from C. corchori inoculum. Three replicates were taken for each treatment. The paired cultures were examined after regular intervals up to 21 days and the nature of reactions noted.

Bioassay of bacterial culture filtrate

The bacteria were grown in Czapek-Dox Medium (CDM) pH 6.5 (Tuite, 1969) for 6 days and the culture filtrates centrifuged at 15,000 r.p.m. for 15 min at 8 °C to remove the bacteria. Fifty percent of the supernatant was sterilized at 15 p.s.i. for 15 min while the rest was cold sterilized by vacuum filtration through a sintered glass filter (G-5). Sterile distilled water and uninoculated medium were used as controls. Fresh conidia of C. corchori were suspended in the test solutions and 0.05 cm³ drops were mounted on clean glass slides and incubated at 30 °C for 24 h following the method described by Purkayastha & Mukhopadhyay (1974). Percentage germination (average of 500 spores) and average germ tube lengths (average of 50 germ tubes) were then estimated.

For growth studies, the bacterium and C. corchori were grown in CDM (pH 6.5) either separately or together. After 8 days, the mycelia were collected,
Micro-organisms of Jute Phyllosphere

Washed free of bacteria, dried at 60° for 96 h and weighed. Bacterial growth was estimated by measuring the optical density (o.D.) of the culture medium in a Corning colorimeter.

Separation of active principle

To isolate the antifungal substance from the culture filtrate of Bacillus megaterium (B-23), 100 cm² of the cell free culture filtrates were extracted with equal volumes of ethyl acetate, chloroform, hexane or petroleum ether. The organic fractions and the corresponding aqueous fractions were evaporated to complete dryness in a rotary evaporator at room temperature (320) and the residue in each case was redissolved in 1 cm³ of phosphate buffer (pH 6-0). To test the effect of the organic solvent residues, similar volumes of organic solvents as used for extraction of culture filtrate, were evaporated and the residues redissolved in 1 cm³ of buffer for use as controls. Fresh conidia of C. corchori were suspended in these test solutions and assayed for the presence of the inhibitory principle (Purkayastha & Deverall, 1965).

Dose response test

The ethyl acetate fraction was dried and the residue dissolved in 1 cm³ of 0-01 M phosphate buffer (pH 6-0). Serial dilutions were prepared in buffer to give concentrations 0-5, 0-25, 0-125, 0-0625 and 0-03125. These were tested on spores of C. corchori (B-23), 100 cm³ of

To study the effect of Bacillus megaterium (B-23) on lesion development by C. corchori, leaves of intact jute plants were sprayed either with the bacterial suspension (in sterile water) or its cell free culture filtrate prepared from 8 day-old cultures on CDM. Sterile distilled water was used as control. Uninoculated nutrient medium was not sprayed as control since it stimulated lesion production and spread. After 24 h treated leaves were detached and 0-01 cm² drops of spore suspension of C. corchori (10 × 10⁶ spores cm⁻²) were placed on the leaves (ten drops per leaf). Percentage production of lesions after 48 h and their spread after 96 h were noted.

RESULTS AND DISCUSSION

Microbes on jute phyllosphere

Of the fungi and bacteria isolated from jute leaves twenty-six strains of fungi and twelve of bacteria were positively identified. Aspergillus and Penicillium spp. were common on leaves but saprophytic deuteromycetes like Phoma, Cladosporium, Alternaria and Helminthosporium and parasites like Myrothecium verrucaria, Colletotrichum corchori and Alternaria citri were also recorded. Among the bacterial isolates, Bacillus spp., both small-celled and large-celled, were abundant. Apart from these, Pseudomonas, Corynebacterium, Micrococcus, Actinobacter and Enterobacter spp. were also present. A black strain of yeasts-like Hormonema of uncertain affinities was also isolated. Most of these microbes were obtained from young leaves but the dark-coloured saprophytic hyphomycetes i.e. Phoma, Cladosporium and Alternaria were usually found on the older leaves.

Paired cultures

Four different types of reactions were observed when the organisms were paired with C. corchori. They were as follows:

(A) Homogeneous. Free intermingling between pairing organisms.
(B) Overgrowth. C. corchori overgrown by the other test organism.
(C) Cessation of growth at line of contact. Two organisms grew towards each other but growth stopped at the common margin.
(D) Aversion. A clear zone of inhibition was observed between the two organisms. The types of reactions observed with the different organisms are summarised in Table 1.

With most fungal isolates, the two fungi grew towards each other and stopped growth at the line of contact. In the case of Gladiolus cryptogynus, the mycelium was raised at the line of contact. In many cases, particularly with the fast growing fungi and bacteria, C. corchori was overgrown by the test organisms. The homogeneous reaction was obtained with most bacteria but with very few fungi. Aspergillus nidulans and Penicillium exalatum exhibited a well marked averted reaction, thus indicating a strong antagonistic reaction. Complete inhibition of growth of C. corchori was discerned when paired with Bacillus megaterium (strs. B-16, B-23). Therefore, two fungal and two bacterial isolates can be regarded as potential antagonists of the anthracnose fungus.

Effect of B. megaterium (B-23) on growth and spore germination

The most antagonistic bacterium in these tests, B. megaterium, was grown in CDM alone and also with C. corchori. The o.d. of the bacterial suspension was 0-45 when grown alone but 0-39 when grown with the fungus. This difference (13-3%) is, how-
R. P. Purkayastha and Bhaswati Bhattacharyya

Table 1. Pairing of Cultures

<table>
<thead>
<tr>
<th>C. corchori with</th>
<th>Type of reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colletotrichum corchori Pangh &amp; Singh</td>
<td>A</td>
</tr>
<tr>
<td>Phoma violacea (Berd.) Eveleigh</td>
<td>A</td>
</tr>
<tr>
<td>Rhizopus stolonifer (Berk. &amp; Curt.) Link</td>
<td>B</td>
</tr>
<tr>
<td>Pencillium spp. (trs. P-1, P-3)</td>
<td>B</td>
</tr>
<tr>
<td>Trichoderma harzianum Rifai</td>
<td>B</td>
</tr>
<tr>
<td>Cladosporium oxysporum Berk. &amp; Curt.</td>
<td>C (mixed)</td>
</tr>
<tr>
<td>Pencillium spp. (trs. P-16, P-21)</td>
<td>C</td>
</tr>
<tr>
<td>Aspergillus spp. (trs. A-7, A-14)</td>
<td>C</td>
</tr>
<tr>
<td>Phoma sorghina (Sacc.) Boerema, Dorenbosch &amp; van West</td>
<td>C</td>
</tr>
<tr>
<td>Corynebacterium corchori Tandon &amp; Bilgrami ex M. B. Ellis</td>
<td>C</td>
</tr>
<tr>
<td>Alternaria alternata (Fr.) Keissler</td>
<td>C</td>
</tr>
<tr>
<td>Pencillium candidum Carter &amp; Thom</td>
<td>D</td>
</tr>
<tr>
<td>Corynesporium sp.</td>
<td>A</td>
</tr>
<tr>
<td>Enterobacter cloacae (Jordan) Hormaeche &amp; Edwards</td>
<td>A</td>
</tr>
<tr>
<td>E. aerogenes (Kraus) Hormaeche &amp; Edwards</td>
<td>A</td>
</tr>
<tr>
<td>Pseudomonas fluorescens (Treviranus) Miguels</td>
<td>A</td>
</tr>
<tr>
<td>Micrococcus luteus (Schroeter) Cohn</td>
<td>A</td>
</tr>
<tr>
<td>Actinobacter solonaeus (Beijerinck)</td>
<td>A</td>
</tr>
<tr>
<td>Baumann, Doudoroff &amp; Stanier</td>
<td></td>
</tr>
<tr>
<td>Bacillus firmus Bredman &amp; Witten</td>
<td>A</td>
</tr>
<tr>
<td>B. pheustae Meyer &amp; Neide in Neide</td>
<td>A</td>
</tr>
<tr>
<td>B. cereus Frankland &amp; Frankland</td>
<td>B</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>B</td>
</tr>
<tr>
<td>B. subtilis (trs. B-16, B-23) De Bary</td>
<td>No growth of C. corchori</td>
</tr>
</tbody>
</table>

Table 2. Effect of cell free culture filtrate of B. megaterium (B-23) on spore germination and germ tube length of C. corchori

<table>
<thead>
<tr>
<th>% germination with S.E.</th>
<th>Av. germ tube length (μm) with S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile distilled water</td>
<td>78 ± 5 ± 2.55</td>
</tr>
<tr>
<td>Culture medium (control)</td>
<td>97 ± 6 ± 1.64</td>
</tr>
<tr>
<td>Culture filtrate</td>
<td>65 ± 2 ± 3.91</td>
</tr>
<tr>
<td>Autoclaved</td>
<td>45 ± 3 ± 3.35</td>
</tr>
<tr>
<td>Cold sterilized</td>
<td>32 ± 3 ± 2.55</td>
</tr>
</tbody>
</table>

ever, not significant. The dry weights of mycelia grown with and without bacteria were 2 and 189 mg respectively. There was practically no growth of the fungus in the presence of the bacterium, the percentage reduction in dry weight being 98.9%. The pH of the culture medium did not change sufficiently to warrant such a change in the fungal growth. This result suggests that a toxic metabolite produced by the bacterium might inhibit the growth of C. corchori.

To verify this, a cell free culture filtrate of the bacterium was tested against spore germination and germ tube growth of C. corchori. The pH values of the test solutions varied from 5.5 to 5.9 so further adjustments were not made. The results are given in Table 2. The percentage reduction (80.2%) in germination and germ tube length (65.9%) in the cold sterilised bacterial culture filtrate in relation to distilled water control lends credence to the possibility that an
Micro-organisms of Jute Phyllosphere

Table 3. Bioassay of solvent extracts of culture filtrate

<table>
<thead>
<tr>
<th>Solvent Extracts</th>
<th>% Germination</th>
<th>Av. Germtube Length (jari)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile distilled water</td>
<td>78.5 ± 1.55</td>
<td>122 ± 1.37</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>97.06 ± 1.03</td>
<td>158.61 ± 1.14</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>92.79 ± 0.64</td>
<td>164.00 ± 2.33</td>
</tr>
<tr>
<td>Hexane</td>
<td>94.70 ± 1.18</td>
<td>157.00 ± 2.03</td>
</tr>
<tr>
<td>Chloroform</td>
<td>88.42 ± 2.09</td>
<td>162.30 ± 2.21</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>90.07 ± 1.30</td>
<td>113 ± 2.64</td>
</tr>
</tbody>
</table>

C. corchori

Table 4. Response of C. corchori to different doses of ethyl acetate extract of culture filtrate of B. megaterium (B-23)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>% Germination Reduction with S.E.</th>
<th>% Reduction in Germination</th>
<th>% Reduction in Germ Tube Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (buffer)</td>
<td>95 ± 1.92</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Undiluted</td>
<td>61.55 ± 3.53</td>
<td>44.50</td>
<td>76.50</td>
</tr>
<tr>
<td>1:2</td>
<td>41.70 ± 3.35</td>
<td>20.20</td>
<td>55.43</td>
</tr>
<tr>
<td>1:4</td>
<td>31.23 ± 3.24</td>
<td>10.50</td>
<td>53.31</td>
</tr>
<tr>
<td>1:8</td>
<td>21.31 ± 3.12</td>
<td>30.90</td>
<td>48.42</td>
</tr>
</tbody>
</table>

Table 5. Effect of bacterial suspension and culture filtrate on lesion production by C. corchori

<table>
<thead>
<tr>
<th>Foliar spray with</th>
<th>% Production of Lesions (48 h)</th>
<th>% Spread of Lesions (96 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial suspension</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>Culture filtrate</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>Sterile distilled water (control)</td>
<td>89</td>
<td>0</td>
</tr>
</tbody>
</table>

Inhibitory substance is present in the culture filtrate of B-23. The active principle appears to be partially thermodstable because autoclaved culture filtrate showed a reduced activity.

Isolation of active principle from culture filtrates

An attempt was made to isolate the active principle from culture filtrates of B. megaterium with four different neutral solvents as described in methods. The ethyl acetate fraction was the most active and reduced germination and germtube length of C. corchori more than any other extract in relation to controls (Table 3). Dose response test

The results indicate that the active principle remains significantly effective up to 0-25 dilution, but its inhibitory action decreases significantly with further dilution.
Effect of foliar sprays on lesion development by C. corchori

Leaves of intact plants were sprayed either with the bacterial suspension or cell free culture filtrate of B. megaterium (B-23) as described in methods. Percentage production of lesions and their spread after 96 h, on the treated leaves is given in Table 5. Thus foliar spray with both bacterial suspension and the cell free culture filtrate reduced lesion production and spread of C. corchori lesions, but the former was more effective in this respect.

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REFERENCES


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BIOLOGICAL CONTROL OF ANTHRACNOSE DISEASE OF JUTE

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The control of plant diseases by chemicals can be spectacular but this is relatively a short-term measure. Moreover, the accumulation of harmful chemical residues sometimes cause serious problems. Biological methods, on the other hand, can be economic, self-perpetuating and usually free from residual side effects.1,3

The jute leaf surface was screened for the isolation of microorganisms antagonistic to the anthracnose fungus Colletotrichum corchori. Leaves were washed in sterile distilled water in a mechanical shaker. The washings concentrated by centrifugation and plated on PDA or NA medium2 in Petri dishes following serial dilution.3 The plates were incubated for 48 hr and 96 hr. On intact jute plants, Bacillus megaterium (str. B-23) completely inhibited the growth of C. corchori. This bacterium, when tested against the jute phyllosphere fungi, showed some tolerance to Penicillium oxalicum (str. PHS-6). A well-marked space of aversion was evident throughout the crop season. These organisms were isolated from jute phyllosphere fungi by similar dual culture methods. The antagonistic reaction was tested in vivo against C. corchori by measuring the percentage germination of conidia and germ tube growth in the cell-free culture filtrate of the bacterium. The bacterium was grown for 7 days in Czapek Dox medium4 and the bacterial cells removed by centrifugation at 15,000 rpm for 15 mins. Fifty percent of the superannuant was sterilized by autoclaving for 15 mins at 15 psi pressure (at 121-6°C) and the rest by a Sintered glass filter (G-5). The filtrate and the autoclaved solutions were tested against C. corchori.4

There was a 80% reduction in germination (17%) of the C. corchori conidia. It appears that the active principle is partially thermolabile.

To isolate the active principle, equal volumes of the cell-free culture filtrate (unheated) were extracted with four different organic solvents - ethyl acetate, chloroform, hexane and petroleum ether. The solvent fractions and the corresponding aqueous fractions were evaporated under reduced pressure in a rotary film evaporator, the residues dissolved in 1 ml of 0.01M phosphate buffer (pH 6.0) and the aliquots tested against spores of C. corchori. The ethyl acetate fraction showed maximum inhibitory activity (94% reduction in germination and 79% reduction in germ tube length) which was evident even after four times dilution. Further dilutions, however, showed negligible activity.

To test the effect of the bacterium on anthracnose disease intact jute plants were first sprayed with either dilute suspension of the bacterium or its cell-free culture filtrate. After 24 hr, selected plants were inoculated with the spore suspension of C. corchori (10 × 10^6 spores/ml) and incubated for 72 hr for lesion formation. Besides, in order to substantiate the results of this experiment, some treated (either with bacterial suspension or culture filtrate) leaves were detached, inoculated with C. corchori and incubated under moist conditions at room temperature (30-32°C). The results of both the experiments are given in table 1.

<table>
<thead>
<tr>
<th>Inoculation Treatment</th>
<th>% inhibition of lesion spread</th>
<th>% reduction of lesion production spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>On detached leaves**</td>
<td>Bacterial suspension culture filtrate</td>
<td>88</td>
</tr>
<tr>
<td>On intact plants*</td>
<td>Bacterial culture filtrate</td>
<td>75</td>
</tr>
</tbody>
</table>

*In relation to distilled water controls.
**Inoculated for 48 hr and 96 hr.
# Incubated for 72 hr and 120 hr.
Thus, *Bacillus megaterium* (B-23) could be used quite effectively for controlling anthracnose disease of jute caused by *C. corchori* (figure 1). The bacterial suspension appears to be more effective than its cell-free culture filtrate probably because the bacteria can multiply rapidly on the leaf surface before inoculation with the test organism. The antagonistic effect is not due to competition for nutrients alone since the culture filtrate can also reduce disease incidence on the leaf surface to a significant extent. In a similar attempt to control anthracnose of cucumber seedlings, Leben and Daft1,2 have used washed cells of *Pseudomonas* sp.

Figure 1. Upper leaves with lesions caused by *C. corchori*, lower leaves treated with bacterial suspension showing a few small lesions.

A-180. The bacterium was sprayed 24 hr prior to inoculation with the test fungus which effectively controlled the disease. Swinburne3,4 also succeeded in controlling leaf scar of apple (caused by *Nectria galligena*) by prior inoculation with the bacterial suspension of *Bacillus subtilis*.

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ABSTRACT

The impact of leaf surface environment on microbial activities has been briefly reviewed. Interactions among phylloplane microbes and their effect on host plants have been discussed in order to explore the possibilities of biological control of various foliar diseases. The effects of air pollutants, pesticides and pollen grains on the phyllosphere microflora have also been emphasized.

INTRODUCTION

The leaf surface constitutes a distinct micro-habitat, the phylloplane, which is inhabited by a large and varied assemblage of saprophytic and parasitic micro-organisms. They interact both among themselves and also with the living plant. The study of the phylloplane is still at its infancy, but the field...
of investigation is intriguing and promising, specially to a plant pathologist for its immense potentiality in evolving new methods for controlling pathogens and pests through biological means. The problem of chemical pollution caused by residual fungicides/pesticides can well be solved by biocontrol methods. Moreover, the interacting organisms in the phyllosphere can induce defensive reactions in the plant effective against disease-causing organisms. These are some of the major aspects of phyllosphere studies.

This review is an attempt to bring together a great variety of relevant findings on phyllosphere research, especially the Indian work has been incorporated here. For the sake of convenience, the review has been divided into a few subdivisions, with a caution that it is the whole, rather than the parts, which is important in our attempt to find out the facts behind this mysterious ecological niche.

**PHYSICAL CHARACTERISTICS OF PHYLLOPLANE INFLUENCING MICROBIAL GROWTH**

The leaf surface is usually covered by a living layer of tissue, the epidermis. The role of cuticle, wax and other substances intimately associated with the epidermis in disease resistance of plants has been discussed by several workers and a few relevant examples are cited here.

Johnston & Sproston (1965) reported that both *Monilinia fructicola* and *Stemphylium sacchariiforme* penetrated artificial collodion membranes as well as separated cherry leaf cuticles. But when cuticle of Gleditsia triacanthos was separated by a pectinase solution and exposed to the said organisms, it was resistant to penetration by the two fungi. The chloroform soluble materials of the cuticle reduced spore germination and germ tube length, but not significantly.

The effect of beet root leaf surface wax on germination of *Botrytis cinerea* spores was studied by Blakeman & Sztejnberg (1973). The wax reformed on glass fiber inhibited spore germination but the rate of inhibition was less than that found on intact beet root leaves. This inhibition could be overcome by addition of nutrients to spore suspensions, or by spraying the leaves with water or chemicals like trichloroacetic acid (TCA) prior to inoculation. Both qualitative and quantitative variations in leaf surface waxes of three varieties of wheat of varying susceptibility to *Alternaria triticina* has been noted by Kumar (1974), the wax content being high in the resistant cultivar, low in the susceptible, and medium in the moderately susceptible cultivar. Also, an acetate of n-hydroxy acid was found only in the wax of the resistant variety. The wax of the susceptible variety was not only deficient in this acetate, but also lacked n-secondary alcohols and an uncharacterised lipid. Blakeman & Atkinsom (1976) have also detected the presence of a spore germination inhibitor in leaf surface waxes of various plants including Chrysanthemum, which inhibited the germination of spores of *Mycosphaerella lingulata*, a pathogen of Chrysanthemum, to a greater extent than spores of *Botrytis cinerea* and *Cladosporium fulvum*, both non-pathogens.

Apart from structural components of the epidermis, certain surface modifications like trichomes and leaf textures also govern the survival and growth of micro-organisms on
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the leaf surface. Yarwood & Gardner (1970) while studying the relative lengths of surface trichomes and conidiophores of powdery mildews observed that the conidiophores were usually shorter than the leaf hairs on the upper surface. On the lower surface, the conidiophores were usually long. The leaf hairs were also longer on the adaxial rather than on the abaxial side. For example, Microsphaera alni growing on Quercus durata, M. alni on Corylus californis, and Uncinula necator on Vitis vinifera showed longer conidiophores on the lower surface, their length being almost double that on the upper surface. It was suggested that conidiophores would be longer on leaves with dense hairy covering than on leaves having sparse hairs. However, notable exceptions are also present.

Rapilly & Foucault (1976) first commented on a common but unobserved phenomenon—the retention of fungal spores by the leaf epidermis. Three levels of retention have been noted on the scabrous surface of wheat leaves; whereas on the smooth horsebean leaf retention was better than on wheat, although statistical differentiation into types of retention could not be made. A high relative humidity raised the retention level in most of the leaves. On smooth leaves, retention depended on the area of contact between the spore and the leaf, while on rough surfaces, an inverse correlation between the level of retention and the speed of spore fall was established. Obviously factors that were unfavourable to spore retention reduced the disease incidence in the field.

CHEMICAL ENVIRONMENT OF LEAF SURFACE

The colonisation of a leaf surface by microbes depends on a number of factors, the most important being the presence of nutrients and absence of toxic materials. The leaching of substances from plant surfaces by rain, dew and moisture films is an accepted and much discussed phenomenon. Various plant metabolites such as carboxylic acids, amino acids and sugars (Tukey & Romberger, 1959; Morgan & Tukey, 1964) and even hormones like gibberellins (Koerl & Tukey, 1968) have been detected in leachates. It appears that any substance present in a plant—be it a metabolite, a storage product, or an extraneous substance, can be leached out from a plant under suitable conditions. The loss by leaching varies with the plant species and can be influenced by light intensity, temperature, the duration of the leaching period, maturity of the leaves, and the general vigour of the plant (Tukey & Romberger, 1959). Leaching has been found to affect the yield and quality of a product, and to increase the susceptibility of plants to pathological and physiological disorders. The substances that are leached out can be recycled, the adjacent plants or the same plant may take up the leached products through the roots. Leaching can also affect the number, nature and the behaviour of phylloplane and soil microbes (Tukey, 1971). Godfrey (1976) reviewing the papers on leaf leachates has commented on the composition of leachates which vary from plant to plant and also from organ to organ.

The effect of aqueous leachates on growth and multiplication of micro-organisms residing on leaf surfaces is well known. About 70% increase in conidial germination of Peronospora parasitica in soybean leaf leachates has been recorded (Mckenzie & Wyellie, 1968). Vario-
tions in effects of exudates from Sorghum varieties of varying susceptibility to the anthracnose fungus, *Colletotrichum graminicola*, were also noted by Sharma & Sinha (1971). A stimulation of spore germination of *Helminthosporium oryzae* in rice leaf exudate was regarded as due to leaching of amino acids onto the leaf exudates (Purkayastha & Mukhopadhyay, 1974). Similarly, a stimulant of germination of conidia of *Colletotrichum corchori* was isolated by Khan & Strange (1975, 1976). Dixit & Gupta (1979) have demonstrated that exudates from a susceptible cultivar of barley could stimulate germination of *Alternaria alternata* spores much more than that from the resistant cultivar. Sometimes, after infection, the leakage of amino acids and electrolytes into the surface fluids increase as reported for *Xanthomonas citri* infection of citrus leaves (Goto et al., 1979).

The presence of antifungal substances in infection droplets has also been the subject of much work and conjecture. In 1967, Deverall reported the presence of an ether soluble antifungal substance in infection droplets containing *Botrytis cinerea* spores, on bean pods. This substance could inhibit growth of germ tubes and also nullify the stimulatory effects of several sugars and amino acids present in the droplets. The presence of antifungal compounds on alfalfa leaves inoculated with *Colletotrichum plumulae* and *Helminthosporium turcicum* and detected by Higgins & Millar (1968). It was suggested that the production of phytoalexins in response to infection by *H. turcicum* and *C. plumulae* could play an important role in the resistance of alfalfa to these and other fungi. In a later publication (1969), the authors reported that *Stemphylium loti* could degrade the phytoalexin produced, while *H. turcicum* could not, thus explaining the pathogenic and non-pathogenic nature of the organisms.

An interesting observation was made by Grover (1971) who studied the effects of host exudate chemicals on appressoria formation by *Colletotrichum pipreatum*. According to him, appressoria formation was dependent on the quality and quantity of nutrients, and the balance between the stimulatory and inhibitory substances present in the infection drop. Farber & Blackman (1978) also reported that leaf surface chemicals affected appressoria formation by *Colletotrichum acutatum*. A survey of the microflora on leaves of some tropical plants indicated a variation both in number and in nature of the microbes present on the different genera studied (Ewaya et al., 1973). These differences were attributed to the presence of antifungal substances diffused out onto the leaf surfaces, which preferentially affected the growth of the different microorganisms. A similar view was put forward earlier by Purkayastha & Deverall (1964) regarding the differential responses of *Botrytis* spp. to bean leaf diffusates. Purkayastha & Ray (1975, 1977) have detected the presence of phytoalexins in jute leaves after infection by *Colletotrichum corchori* and have discussed its possible role in lesion development. The leaf surface environment of *Acer platanoides* was found to be unfavourable for growth of phylloplane fungi during the summer months (Irvine et al., 1981). The inhibitory activity of leaf washings reduced as the season advanced, and this could be correlated with an increase in the phyllosphere microbial populations. A stimulation of conidial germination
of *Colletotrichum gloeosporioides* on host surface, and an inhibition on non-host surface has been recorded by Purkayastha & Sengupta (1973). The production of infective structures on rice leaves by *Cochliobolus miyabeanus* was correlated with the activity of certain leaf cells. Chemical stimulation or inhibition of appressoria formation was due to two different types of cells (Hau & Rush, 1979).

It appears from the above statements that the microbes sometimes have to colonise and live on a potentially hostile environment which erects both structural (mechanical) and chemical barriers that have to be surmounted before a population can establish itself. Though the leaf surface also supplies nutrients to its microflora, the competition for this becomes too great to be of any use to a colonising organism. The inhibitory substances sometimes leach out with the nutrients and as a result, the latter cannot stimulate the growth of micro-organisms. However, further work may elucidate the exact role of the leaf surface in controlling its resident microbial populations.

**BIological Environment of the Phylloplane**

Leaves and other aerial parts of plants are invariably colonised by a variety of epiphytic micro-organisms, and their survey and identification have been a major field of study for plant pathologists. The leaf surface microbes can be broadly classified into four main groups: (a) Parasites, (b) Saprophytes, (c) Microbial epiphytes and (d) Symbionts.

(a) Parasites are organisms that depend on the living leaf for their nutrition and cause harm to the plants. Sharma & Mukhopadhyay (1974 a, b) recorded the higher incidence of some pathogenic species of *Candida, Mucor*, *Colletotrichum, Fusarium* and *Phoma* on mature leaves of *Sesamum orientale* and a progressive decrease on senescing ones. The conidial germination of *Erysiphe graminis* f. sp *hordei* on the basal and middle portions of adaxial surfaces of barley leaves was also higher than that on the corresponding portions on the abaxial surface, as reported by Russell et al. (1975). The stomatal behaviour and related phenomena were compared before and after infection of leaves of *Pisum sativum* by *Erysiphe pisi* (Ayres, 1976). It was interesting to note that the stomatal opening was more on the infected leaves than on healthy ones. Also, after a few days, stomatal movement ceased altogether and the stoma remained open all the time.

(b) The saprophytes resides on the dead areas of the living leaf, or colonise the leaf when it is desiccating or senescing. Among the phyllosphere microflora, the saprophytes form the major bulk, and consequently more studies have been carried out on the saprophytic flora of leaves.

Freind (1965) has isolated and identified the sooty moulds on the leaves of lime trees. Two fungal species namely *Aureobasidium pullulans* and *Cladosporium herbarum* were common, but *Fumago* spp. and members of the Capnodiales, which are usual components of the sooty moulds, were not found on lime. Last & Deighton (1965), surveying the non-parasitic microflora on surfaces of living leaves in the temperate regions, concluded that foliage microflora consisted primarily of bacteria, and yeast-like fungi. In the tropics however, algae, lichens and ascomycetes predominated.
The bacteria, usually coloured, included the pseudomonads, xanthomonads and Flavobacterium sp., while the yeasts were usually members of the Cryptococcaceae and Sporobolomyces. The number of microbial colonies increased with the age of the leaf and were usually less in winter than in summer. Fungal populations varied on the two surfaces of the leaf, the member of Sporobolomyces were more abundant on diseased than on healthy leaves. Mukherjee & Sharma (1972) have recorded the changes in mycoflora associated with leaves of Sesamum orientale under varying atmospheric conditions. Kumar & Gupta (1976) reported that on potato the population of microbes varied with the variety, maturity of leaves, age of plant and climatic conditions. The non-parasitic mycoflora on living leaves was classified by Garg et al. (1978) as casuals and residents. The different plant species supported distinct fungal populations, chiefly of casuals. The dominant residents were more or less similar for the three plant species studied —namely barley, triticale and egg-plant. The fungal population of leaf surfaces of Brassica campestris, has been investigated by Grover et al. (1979) who isolated 29 spp. of fungi from leaves at various developmental stages. The maximum population was recorded at the flowering stage. The microflora of healthy and powdery mildew-infected leaves of barley was compared (Sharma & Garg, 1979). Infection caused a replacement of one group of fungi restricted to healthy leaves. Thus, the infected leaves represented an ecological niche different from the non-infected leaf phylloplane. Investigations on the microflora of Acacia nilotica and Psidium guajava leaves have revealed a close correlation between the phyllosphere flora and the aerospora, though all fungi present in the air could not be detected in the phylloplane (Saxena & Saksena, 1982b).

In 1970, Mishra & Srivastava noted that in Triticum aestivum, the saprophytic phylloplane flora varied with the age of the host plant, and also with the colour of the leaves. Similar studies on mycoflora of living and dead leaves of Nothofagus truncata revealed that young leaves acquired a mycoflora of internal parasites and discrete surface colonies soon after unfolding (Ruscoe, 1971). These were succeeded by fungi imperfecti and ascomycetes. Many fungal colonies became either epiphyllous or hypophyllous with time. The green and yellow leaves of Hordeum vulgare showed different species in their microfloral flora (Mishra & Srivastava, 1974). Foliar spray with different concentrations of three amino acids and an organic acid stimulated the growth of a few selected species of micro-organisms on Hordeum leaves. More than 30 genera of fungi were isolated from leaves of two varieties of rape-seed (Tsuneda & Shoropad, 1978). There fungi were classified into three groups depending on their occurrence—those which developed with onset of senescence, or occurred rarely, or were present throughout the growing season. A survey of leaf inhabiting fungi of Quercus robur (Cox & Hall, 1978) showed that the population varied between trees, increased towards the end of the season and occurred more profusely on the upper surface than on the lower. The maximum colonisation on potato leaves occurred in autumn when the lower surface harboured a larger number of microorganisms than the upper surface (Kumar & Singh, 1981). It has been reported that the
age of the cultivar, sowing time and soil amendment by fertilisers affect the phyllosphere microflora of rice plants (Sarkar & Samaddar, 1983). In general, 90 days old plants had high population of micro-organisms irrespective of sowing time. The leaf surface bacterial populations increased with increase in the doses of organic and inorganic fertilisers. An interesting observation was made by Saxena & Saksena (1982 a), who found that leaves of Trapaeolum majus infected with Laurellula taurica contained a distinct type of mycoflora which was absent in the healthy leaves.

(c) The epiphytes normally occupy the leaf surface as their natural habitat, and they grow and multiply on apparently healthy leaves. In most cases the epiphytes utilise the exudates or diffusates of leaf. However, they do not harm the living plant.

According to Leben (1965) pathogens are often subject to diverse influences in a complex microbial ecosystem which fluctuates both qualitatively and quantitatively with age and condition of the plant, influx of foreign materials, and with changes in environmental conditions. Actively growing tissues were first colonised by bacteria, followed by fungi, yeast-like fungi and ultimately the yeasts. The pathogenicity of a fungus appeared to be governed by its ability to compete successfully with the epiphytic organisms for nutrients and space, and its ability to degrade any antifungal substance produced by the epiphytes.

The composition of epiphytic yeast flora on crops and woody plants normally changed with the seasons (Krasnykov et al., 1971). Pullularia spp. prevailed in summer followed by about 40 other spp. including Rhodotorula and Cryptococcus which became dominant in autumn. Yeast flora was minimum in spring and maximum during the period of flowering, probably due to the presence of extra nutrients provided by the pollen and necrotic portions of senescing leaves. The number of cells on wet leaves became almost four times that found on dry leaves of wild woody plants.

Young (1978) has studied the survival of epiphytic bacterial species of Pseudomonas, Xanthomonas and Erwinia groups on leaves of Prunus. These organisms usually exist in well protected sites in the leaves out of reach of common fungicidal sprays.

(d) The symbionts as the name signifies, enjoy a 'give and take' relationship with the living plant. Researches on phyllosphere symbionts have been rare, and only a few cases are discussed here.

Among the varied assemblage of leaf surface microorganisms there are some which can fix atmospheric nitrogen. Ruinen (1956) first reported the presence of Beijerinckia, a N₂-fixing bacterium, on leaf surfaces of many tropical trees. The bacteria absorbed nutrients from the leaf debris, and the plant used the N₂ fixed by the organism — and thus a symbiotic relationship was established. This bacterium was also found in 95% of the leaves surveyed in Indonesia (Ruinen, 1974, 1975).

Many tropical plants also carried a few species of Azotobacter, another well known N₂-fixing organism. Even conifers like Pseudotsuga douglasii carry N₂-fixing microbes in the needles (Jones, 1976). These bacteria come in contact with the plant by accident as dust from forest roads or as airborne or splatter infections, and their distribution on the leaf surface is quite random.
The symbiotic relationship between the N₂-fixing organisms on the phyllosphere and the leaf can be explained as follows. The leaf surface provides moisture (from dew) and nutrients (from exudates) to the bacterium. The organism, in exchange, furnishes the plant with soluble nitrogenous compounds which are absorbed through the leaf. Thus a closed cycle of foliar feeding is established. In many cases, the soluble nitrogen fixed on leaf surface is washed down by rain and is cycled through the soil and root system of the plant. This constitutes an open cycle whereby other plants may be benefited by taking N₄ from the soil.

Another interesting feature in this connection is the fixation of N₂ on the leaf surface by leaf nodule bacteria. The presence of leaf nodules in about 400 tropical and semitropical species of Myrsinaceae and Rubiaceae has been reported (Fletcher, 1976), but studies have primarily been concentrated on Ardisia (Myrsinaceae), Pavetta and Psychotria (Rubiaceae). The plants that are usually nodulated often do not survive if deprived of the bacterium. However, the basis of this obligatory symbiosis is not yet clearly known. Burris (1977) has observed that very young Ardisia plants are colonized by the bacteria, and plants devoid of the same do not grow well. The presence of Klebsiella on the lower surface of Psychotria leaves has been a point of controversy for years. Silver et al. (1963) have claimed that this bacterium can fix atmospheric N₂, whereas Becking (1974) did not find any evidence of the same. Bacteria are also found in the leaf glands of Dioscorea sp. but their role in N₂ fixation is not known (Burris, 1977).

Though it is now well accepted that microorganisms do fix atmospheric N₂, in the phyllosphere, it is difficult to quantify the amount fixed because the fixation often depends on a variety of factors including humidity of leaf surfaces and changes in microbial populations after a heavy shower. These may change the quantity of N₂ fixed by the bacteria. However, it is presumed that N₂-fixing bacteria may account for about half of the total amount of N₂ fixed annually (Shanmugam & Valentine, 1975). In future, bacteria may be "tailored" genetically for fixing of nitrogen on a variety of plant surfaces.

INTERACTIONS OF PHYLLOSHERE MICRO-ORGANISMS

In 1955, Wood & Tveit suggested methods of controlling plant diseases by using antagonistic organisms. The papers pertaining to interactions of surface microbes have been reviewed by Sinha (1965). He observed that leaf exudates could affect the surface microbes directly, which in turn could interact with the invading organisms. Some of the surface organisms were hyperparasites growing parasitically on the intruder. Others affected the activity of the pathogens indirectly by inducing the formation of fungistatic substances by the host plants. Direct antagonism by competing for space and nutrients was very common. The pathogens themselves produced self stimulatory and self inhibitory substances bringing about associative or antagonistic effects. A study of such interactions could ultimately lead to the formulation of biological methods of plant disease control.

There has been repeated attempts to control growth of fungal pathogens by antagonistic bacteria, yeasts or fungi, and the literature pertaining to these studies have been
reviewed by Skidmore (1976). The mechanisms underlying the antagonistic reactions vary, and some may also overlap. For example, antagonism by competition for nutrients may be present along with direct parasitism of one organism by another, or pH effects may overlap toxic activities of metabolites of the antagonist, and so on. The main problem connected with biological control appears to be the specific qualities required by a potential antagonist, namely, it should be reliable, economic, and non-phytotoxic. The availability of these qualities in one organism may be very rare. Extensive screening is required before an organism is recommended as a measure of biological control.

The interactions between micro-organisms may be categorised as follows: (a) fungus vs fungus, (b) fungus vs bacterium and (c) bacterium vs bacterium.

(a) Fungus vs fungus

Among the phenomena of microbial interactions, the fungus vs fungus interactions were studied first. Bhatt & Vaughan (1963) showed that fungi isolated from strawberries varied in their ability to inhibit *Botrytis* sp causing fruit rot, the variations ranging from no effect on each others growth to mutual inhibition. Hyperparasitism, i.e. parasitism of one fungus on another has been studied in detail by Burnett (1963, 1964), Boosalis (1964) and Renato (1977). This parasitism has been found to be similar to the parasitic relationship between micro-organisms and higher plants.

Species belonging to fourteen genera of fungi were isolated from tobacco leaves by Chauhan & Grover (1973) who screened their antagonistic properties against *Helminthosporium* epiphyllum. Some fungi including *Blakeslea, Mucor, Aspergillus, Cunninghamella, Thielaviopsis* and *Colletotrichum* could exert their concerted antagonism against the test pathogen. Interactions between *Alternaria pisi* and saprophytic flora of onion leaves were investigated by Fokkema & Lorbeer (1974). The common phyllosphere saprophytes *Aureobasidium pullulans, Sporobolomyces roseus*, etc. reduced superficial mycelial development of the fungus. The antagonism was not due to changes in leaf surface pH or depletion of carbohydrates and amino acids from the phyllosphere. The influence of *Brassica* leaf saprophytes on *Alternaria brassicae* was due to antagonisms by *Aureobasidium pullulans* and *Epilobococcus niger* (Pace & Campbell, 1974). The inhibitory effect of these two organisms was greater when introduced to the plant before inoculation with the pathogen. Thirty eight fungi occurring on the phyllosphere of *Cicer arietinum* L. were screened for their antagonism against germination of *Uromyces* sp. (Sinha & Bahadur, 1974). It was confirmed that the phyllosphere fungi produced certain inhibitory substances which caused the reduction in germination of rust spores. The role of leaf surface yeasts and bacteria in reducing disease caused by plant pathogens has been emphasized by Fokkema (1978). According to him 'phyllosphere fungi behave as scavengers on leaves with enhanced nutrient conditions (e.g. by pollen, or honey dew) diminishing the nutrient availability to pathogens. The yeast flora of field grown wheat was manipulated by spraying cell-suspensions of common yeasts- *Cryptococcus awntii* var. *flavescens* and *Sporobolomyces roseus*, along with
nutrients, which caused an approximately ten-fold increase in yeast populations within a week. Inoculation of plants having enhanced yeast flora with *Septoria nodorum* and *Cochliobolus sativus* showed a 50% reduction of disease incidence from that on control leaves having normal yeast flora (Fokkema et al., 1979). The antagonistic activity of some leaf surface fungi against *Alternaria brassicae* and *Drechslera graminis* has been demonstrated by Rai & Singh (1980). The most antagonistic fungi were *Epicoccum purpurascens*, *Aureobasidium pullulans* and *Cladosporium cladosporioides*. Sunflower blight caused by *Alternaria alternata* could be controlled by common leaf surface fungi like *Aspergillus niger*, *Trichothecium roseum*, *Penicillium simplicissimum* and *Fusarium semitectum* (Gupta et al., 1981). Bhattacharyya et al. (1981) reported that *Macrophomina phaseolina* infection of jute could be partially controlled by *Aspergillus versicolor*, an antagonist of *M. phaseolina*.

A very interesting and significant phenomenon was noted by Richmond et al. (1979). They found that cucumber plants were systemically protected from infection by *Colletotrichum lagenarium* after previous infection with the same pathogen. The protection by previous infections appeared to be confined to the surface layers because the degree of protection was reduced after removal of the leaf epidermis.

**Fungus vs bacterium:**

Epidermal bacteria can suppress the activities of pathogens causing foliar diseases. Singh & Sinha (1962) showed that out of thirteen fungi and eleven bacteria, isolated from surfaces of brinjal, bottle gourd and lady’s finger and screened for mutual antagonism, four cases were positive. Cucumber seedlings were protected from anthracnose disease caused by *Colletotrichum lagenarium* by spraying the plants with washed cells of isolate A-180, an epiphytic bacterium of cucumber leaves (Leben & Daft, 1964, 1965). Some species of *Pseudomonas* antagonistic to the anthracnose fungus have been used to minimise the intensity of anthracnose of melons growing in the laboratory and in the greenhouse (Panteleev & Budagasyan, 1972). Washed cells of *Bacillus subtilis* were also employed to control canker caused by *Nectria galligena* after leaf fall (Swinburne, 1973, 1978). Hegde et al. (1980) have used the same bacterium to control foot rot of wheat.

Phylloplane bacteria of rice reduced the intensity of blast, brown spot and bacterial leaf blight diseases (Jagadeesh et al., 1978). Chandravathani & Rao (1981) have demonstrated that leaf blight disease of maize caused by *Bipolaris turcica* could be reduced by leaf surface micro-organisms. A high degree of antagonism was detected with the bacterial isolates of *Pseudomonas aetocollagensis* and *Streptomyces sp.*. *Alternaria* blight of chilli was partially controlled by both *Trichoderma viride* (83.5% reduction) and *Streptomyces sp.* (79.5% reduction) as shown by Tyagi & Chauhan (1981). Similarly *Bacillus megaterium* was found to be effective in controlling anthracnose of jute caused by *Colletotrichum cochlioi*. This bacterium reduced the germination of conidia of *C. cochlioi* to a significant extent, thus reducing its pathogenicity. Moreover, the bacterium also secreted a toxic metabolite into the surrounding medium which was inhibitory to the growth of the fungus (Purkayastha...
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& Bhattacharyya, 1982). Normally however, epiphytic bacteria cause an increase in the pathogenicity of the anthracnose fungus by competing for nutrients and creating a starvation situation in which appressoria are rapidly produced on short gram tubes giving rise to infection pegs (Blakeman, 1978).

A study of phylloplane fungi in relation to Xanthomonas malvacearum infections of cotton revealed that increased infection always caused an increase in the number of fungi on the leaf surface (Wadje & Deshpande, 1979). The dominant fungi were found to be Cladosporium, Phoma and Alternaria and it was also noted that the first two fungi along with Curvularia and Aspergillus inhibited the growth of the pathogenic bacterium.

(c) Bacterium vs bacterium

The antagonistic activities of one bacterium against others were first recorded by Goodman (1965, 1967) who isolated a yellow Erwinia like bacterium from apparently healthy buds of Jonathan apple, which checked the infection caused by Erwinia amylovora. Screening of the saprophytic flora of cherry leaves revealed the presence of an Erwinia-like organism which reduced the incidence of leaf scar infections incited by Pseudomonas morsprunorum (Crosse, 1965). While studying the bacterial disease of cotton caused by Xanthomonas malvacearum, Verma & Singh (1976) Verma et al. (1978) observed that avirulent strains, heat killed cells and phylloplane bacteria such as Flavobacterium sp. and Aeromomas sp. could reduce the disease incidence, if inoculated 8h to 48h before infection.

Bacterial leaf streak of rice caused by Xanthomonas translucens f. sp. oryzae could also be controlled by periconulation with Pseudomonas sp., Erwinia sp. and Aspergillus sp. isolated from rice leaves. Pseudomonas and Aspergillus caused total inhibition of growth of the pathogen but with Erwinia symptoms developed although the length and number of lesions were much less than that of controls (Rao et al., 1978).

The above statements indicate that there are ample scopes for isolation and exploitation of a specific organism, be it a fungus, or a bacterium, as an antagonist against many virulent plant pathogens. However, there are very few examples in which introduction of an antagonist has reduced disease incidence under field conditions. A major problem still unsolved is how to apply safely 'avirulent' organisms related to pathogens but which stimulate host resistance. The potential of this method is enormous, but so are the risks. Who can predict that the 'avirulent' organism will not be pathogenic to another plant or will not act synergistically with other pathogens? This is a question put forward by Wilson (1978) which has to be answered before biological control of pathogens can be undertaken under field conditions.

INFLUENCE OF POLLEN ON LEAF SURFACE MICROBES

Pollen grains on leaves sometimes affect microbial populations and their growth. Myra Chau Choa & Preece (1968) recorded a stimulation of spore germination and lesion production of Botrytis cinerea on leaves. The aqueous diffusates of pollen grains were also effective, frozen pollen being more stimulatory than fresh pollen. Moreover, the viability of old spores could be restored by pollen or pollen
diffusates. Though pollen diffusates contained simple sugars like glucose and fructose, neither of them either individually or jointly could simulate the activity of pollen grains.

The effect of pollen on phyllosphere saprophytes and on infection of rye leaves of *Helminthosporium sativum* was investigated in detail (Fokkema, 1971). The saprophytic population on leaves with pollen was much higher (3-20 times) than on leaves without pollen, their number depending on the organisms concerned and the seasons. Addition of pollen/pollen diffusates to spore suspensions of *E. sativum* and *Septoria nodorum* during inoculation greatly increased the severity of disease caused by the two fungi. Under field conditions, inoculation of rye leaves with *H. sativum* soon after flowering caused severe infections due to the presence of pollen grains on leaves. Further addition of pollen, however, did not affect disease incidence probably because the pollen also caused an increase in the phyllosphere saprophytic population which could become antagonistic to the pathogen. Fokkema (1973) has studied the role of saprophytic fungi as antagonists of *H. sativum* both *in vitro* and *in vivo*.

The effect of pollen on microflora of sugarbeet leaves and on infection by *Phoma betae* was studied by Warren (1972). Results revealed that the natural pollen from sugarbeet plants increased the number of microorganisms on the leaf surfaces. When pollen of rye plants were placed on sugarbeet leaves, instead of sugarbeet's own pollen grains, the development of natural microflora was found to be negligible. On the other hand, rye pollen considerably increased the pathogenicity of *Phoma betae* when added to the inoculum on sugarbeet leaves.

Tyagi & Chauhan (1982) have also studied the effect of pollen grains on leaf surfaces of chilli and brinjal in relation to *Alternaria solani* infections. Pollen diffusates were always more effective than natural pollen in increasing fungal infections. The diffusates could also reduce the time required for spore germination.

An interesting relationship existing between pollen grains and a fungus *Retiarius* Gen. nov. was reported by Olivier (1978). Two fungal species *viz.* *R. bovicornutus* and *R. superficiaris* occurring in the phylloplane of some evergreen trees in South Africa, were found to be parasitic on wind-borne pollen grains. The pollen were captured by short spike-shaped hyphae of the fungus, and the latter survived the dry summer months within the pollen grains as thick-walled mycelia. Thus the pollen grains served a dual purpose by providing nutrition and protecting the fungal parasite from desiccation.

From these studies it becomes evident that the pollen grains basically encourage the growth of microbes, mostly by providing nutrition to leaf surface saprophytes. According to Fokkema (1973) and Warren (1972) these pollen grains could stimulate the growth of parasites, saprophytes or epiphytes, and any selectivity in this stimulation can well lead to a means for exploiting them as biocontrol agents with very little side effects.

**FUNGICIDES AND PESTICIDES AFFECTING MICROBIAL COLONISATION**

The leaf surface microbes vary in their responses to extraneous substances such as
fungicides or pesticides. A chemical which is fungicidal to one species may be just fungistatic to other or may have no action at all on the third. These varied responses of micro-organisms can be regulated by changing the appropriate chemical or their concentrations.

The effect of different fungicides in varying combinations on common foliar pathogens of cucumber plants was tested by Campacci et al. (1963). It was noted that 0.25% Copranto, 4% Ziracuivre and 0.5% Ytigran Axul gave the best results. Chauban & Grover (1973) observed the effects of foliar sprays with Zineb and Copper oxychloride on tobacco cultivars for the control of Helminthosporium spiciferm infections. Copper was found to be more effective and persistent on leaves than Zineb. Moreover, the resistant cultivars could retain copper residues more effectively than the susceptible ones. The effects of Captan, Dichloran, Thiram and Verdasan were compared on the germination, cellulose decomposition and starch hydrolysing properties of some common phylloplane fungi by Kuthubuthieen & Pugh (1978). Higher concentrations of the fungicides were required to reduce the efficiency of the fungi for starch hydrolysis and degradation of cellulose than to reduce mycelial growth or spore germination. Captan and Dichloran were less effective than Thiram and Verdasan. The effect of different concentrations of Captan, Ziram, Streptomycin and Penicillin on microflora of wheat and barley leaves were recorded (Mishra & Tewari, 1979). The bacterial population decreased with the antibiotics but were less affected by the fungicides. A combination of fungicides suppressed fungi on Poa pratensis leaves and stimulated bacteria and actinomycetes much more than the individual toxicants (Smiley & Craven, 1979). Sometimes the effects of the fungicides were indirect, as they increased the acidity thus leading to an inhibition of microbial growth.

The mode of action of chemicals other than a few known fungicides on phyllosphere micro-organisms is still obscure. Some reports, however, have indicated an increase in the susceptibility of plants treated with pesticides like DDT. Further studies are required to confirm the results (Tarr, 1972).

AIR POLLUTANTS AND LEAF SURFACE MICRO-ORGANISMS

Very little is known to date about the effects of atmospheric pollutants on the leaf microflora although much has been surmised about their influence on higher plants and animals.

The interactions between Phaseolus vulgaris and Colletotrichum lindemuthianum, causing anthracnose, were altered by treatment of inoculated plants with 15% CO2 (Arnold & Rahe, 1976). A shift towards resistance occurred when plants were placed in CO2 during early stages of fungal penetration. A severe effect was noticed when CO2 was applied at an advanced stage of infection.

The effects of concentration, exposure time, temperature and relative humidity, on toxicity of SO2 to Botrytis cinerea spores were recorded by Couey & Uota (1961). The pollutant toxicity increased proportionately with a rise in relative humidity. SO2 concentration, time of exposure and for every 10°C rise in temperature from 0-30°C. Similarly, inhibition of spore germination of Alternaria
was found to be directly proportional to the \( \text{SO}_2 \) concentration and exposure time (Couey, 1965). Conidial germination of *Diplocarpon rosae* was also inhibited by 35 ppm \( \text{SO}_2 \) in water, the inhibition was permanent after exposure for 3h. Infection of rose leaves by *D. rosae* decreased when plants were exposed to \( \text{SO}_2 \) (1000 ppm) for two days (Saunders, 1966).

The effect of low level ozone fumigation on *Puccinia coronata* causing crown rust of oats was studied by Heagle (1970). Production of uredia was significantly reduced on exposure of the host to 10 ppm \( \text{O}_3 \) for 6 h in light for ten consecutive days after inoculation. Manning & Papia (1972) have also noted the effect of long term low levels of \( \text{O}_3 \) on leaf surface microflora of *Phaseolus vulgaris*. The differences in the microbial populations on leaves of plants grown in ambient air and that containing 6 ppm \( \text{O}_3 \) for 8 h/day, were more quantitative than qualitative.

The leaf surfaces of city trees usually carry particles of trace metals like aluminium, manganese, iron, nickel, lead and zinc. The effects of these metal pollutants on leaf pathogens including *Aureobasidium pullulans*, *Chae­tomium* sp., *Gnomonia platani* and *Pleurophoma* sp. were tested in vitro by Smith et al. (1976). With low doses, none of these metals were inhibitory to the fungi excepting *Chae­tomium* which was inhibited at low doses of zinc, aluminium and iron became fungitoxic at high doses. Thus a direct inhibition of fungal growth on leaf surfaces by trace metal contaminants appear to be unlikely.

Smith (1976) reviewing the literature dealing with the effects of air pollutants on leaf surface microbes and their interactions with the host has shown that only the organisms living on the interface of atmospheric and vegetative compartments of the leaf receive maximum exposure to the pollutants. Parasitism can be either increased or decreased through direct effect of the contaminant on the parasite itself (Heagle, 1973), or the pollutant can alter the disease reaction of the plant by inducing changes in the plant itself. In fact, green plants appear to be much more sensitive to air pollutants than the resident microbes. Most phytotoxic pollutants injure leaves (Pell, 1978) releasing nutrients to the surface which may cause an increase in the saprophytic microflora.

### CONCLUDING REMARKS

There has been an explosion in our knowledge of leaf surface micro-organisms, their interactions and survival on a potentially hostile environment since Ruinen (1956) first drew attention to this area of microbial ecology. However, many questions still remain unanswered. Why does a resident saprophyte prefer a particular host? How do saprophytes behave with each other? Are microbial epiphytes beneficial or harmful to the host on which they grow? Can biological control methods really obliterate the problems of using chemical pesticides? Or will its limitations outweigh the advantages of implementing such methods? In this brief resume attempts have been made to discuss a few interesting observations with the hope that more and more potential researchers will be interested to find the answers to the questions phrased here and thus enrich our knowledge about this mysterious area of microbial ecology.
REFERENCES


Blakeman, J.P. and Atkinson, P. 1976. Evidence for a spore germination inhibi-


Crosse, J.F. 1965. Bacterial canker of stone fruits. VI Inhibition of leaf scar infection of cherry by a saprophytic bacterium from leaf surfaces. Ibid. 56: 149-160.


Khan, S.R. and Strange, R.N. 1975. Evidence for the role of a fungal stimulant as a determinant of differential susceptibility...
of jute cultivars to Coelortrichum corchori. Physiol. Plant Pathol. 5: 157-164.


Kumar, S.N.H. 1974. Qualitative and quantitative variations in leaf surface wax in three varieties of wheat varying in susceptibility to blight caused by Alternaria triticina. Indian Phytopathol. 27: 508-513.


Richmond, S., Kuc, J. and Elston, J.F. 1979. Penetration of cucumber leaves by Colletotrichum lagenarium is reduced in plants systemically protected by previous


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