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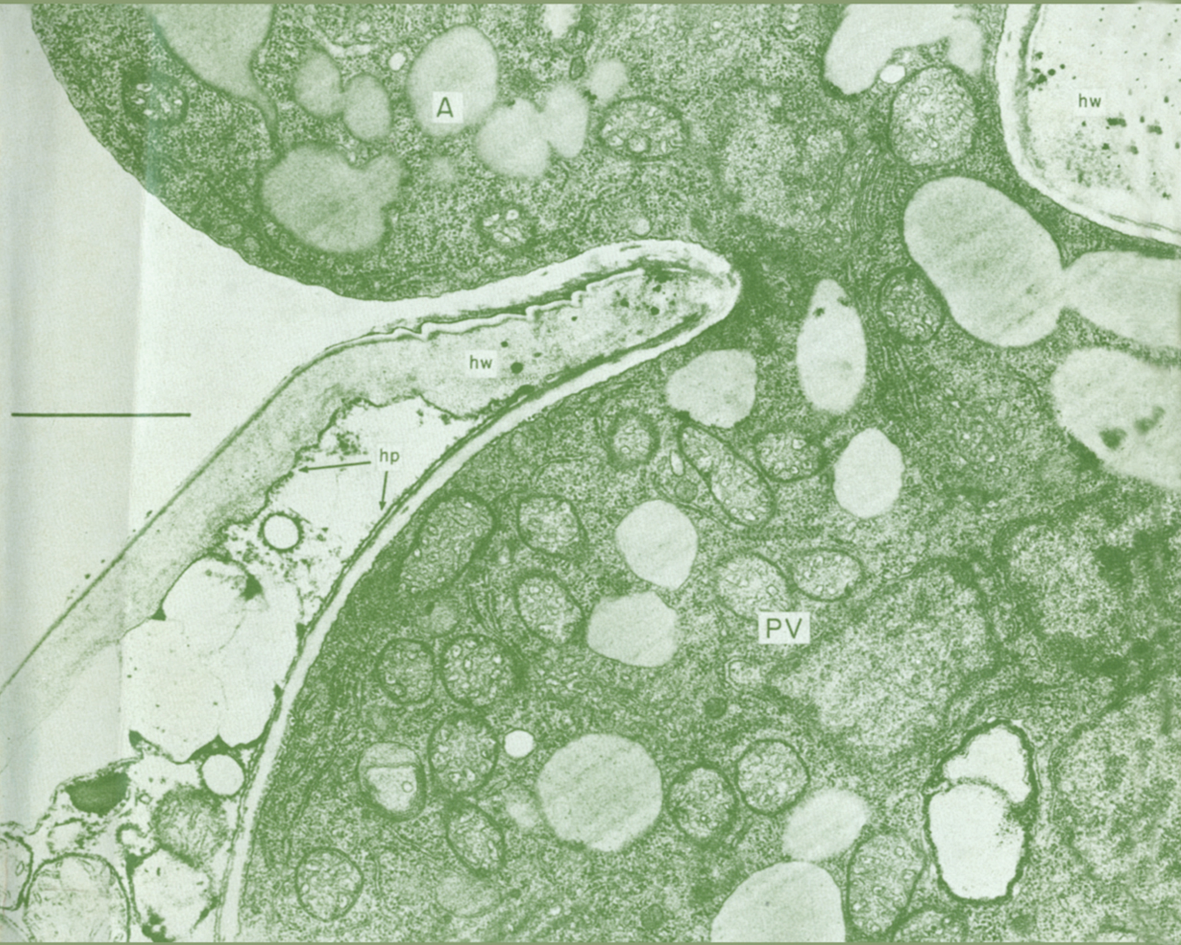

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Biochemical Aspects of Plant-Parasite Relationships

edited by
J. Friend and D. R. Threlfall

Biochemical Aspects of Plant–Parasite Relationships

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Preface

In recent years there has been increasingly more emphasis on the use of biochemical methods and techniques for research in plant pathology and it has now become possible to offer biochemical explanations for several phytopathological phenomena.

It was the intention of the organizers of the Phytochemical Society Symposium held at Hull in April, 1975, on Biochemical Aspects of Plant Parasite Relationships to display some of the more biochemical of the recent research, particularly on the mechanisms involved in the invasion of plants by pathogens, the production of disease symptoms, and the mechanisms involved in the resistance of plants to the invading microorganisms.

Papers on the genetics of fungal–plant interactions and on structural features both of infection and of resistance are included in the volume. The reason for this inclusion is that there has been a tendency for plant biochemists to neglect both structure and genetics as aspects of their investigations and yet it is often through an understanding of the structural and genetical basis of the plant–parasite interaction that a sensible biochemical explanation can be given.

For various reasons it was not possible to produce this volume as soon after the Symposium as has been common for previous volumes of proceedings of the Phytochemical Society. However the authors have revised their manuscripts during the period of enforced delay.

October, 1976

J. FRIEND
D. R. THRELFALL

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CHAPTER 1

Some Observations on Leaf Surfaces During the Early Stages of Infection by Fungi

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I. INTRODUCTION

It is sometimes difficult for plant pathologists to see relevance to controlling plant disease in elegant and detailed basic studies of particular host–parasite interactions, perhaps concerned with membrane damage or changes in the nucleic acids of host plants. I personally accept without reservation the definition of plant pathology given by Moore (1949), which is that it is the job of plant pathologists to "influence the practices of crop husbandry". Crop husbandry goes on in fields, orchards, forests and market gardens. Although more protected kinds of crop husbandry are found in glasshouses and special structures such as mushroom houses, it does not take place in laboratories. One way of reminding ourselves of the essentials of our subject is to look at diseased plants in the field, and in particular to examine, microscopically, the pre-penetration stages of disease, under field conditions. We would then be assured that we know more of the disease before trying to understand it and before attempting control measures.

However, that is not to say that current effort in physiological and biochemical plant pathology is not essential for further progress. Of course it is; what is more, some of our models of the host–parasite interaction are in an exciting stage of development at the present time, as the other contributions in this volume will testify.

II. The Pre-penetration Stages of Infection of Leaves

Almost all of the current basic work in plant pathology is concerned with events which occur *after* parasites have penetrated their hosts. *Before* these later stages of disease can occur, the early pre-penetration stages must be successful. Earlier plant pathologists, such as Marshall Ward (Large, 1940) clearly grasped, and exploited, the fact that during the arrival, adhesion and external growth stages fungal spores on leaves are the most vulnerable to changes in the environment and in particular to the action of fungicidal sprays. The spore of a pathogenic fungus arriving at a leaf surface is a living organism in a most delicate phase of its life history, struggling for existence. It eventually has to make contact with host cell membranes if it is to achieve the possibility (at least) of a more compatible, safe environment; alternatively, for the expression of the host genotype–fungus genotype interaction.

What factors interplay in microevents prior to penetration? Do the events on leaf surfaces differ from the early stages of growth from fungal spores on artificial surfaces? We, as good experimental plant pathologists, use apparently clean fungal spore suspensions and leaves grown in closely controlled environments. But the leaves of crop plants in the field exposed to the weather are much more "messy" than the simple models we may have in our minds (Preece, 1963). The natural history of field infection, and in particular of the pre-penetration stages of infection, is complex, and awkward to handle experimentally, but are we "missing the wood whilst looking at the trees"? This is an extreme suggestion, but, if it generates observation followed by experiments, then it is justified. Our minds are conditioned. Two things especially have helped to induce the idea that the early microscopic stages of infection in the field do not need modern work. These are (1) the comforting generalized notion of a "typical" infecting spore, and (2) the ease with which many spores germinate in water on glass slides. It is a fact that what we know of the earliest stages of infection in particular diseases—the pre-penetration stages—often depends on a single drawing or photomicrograph in a paper concerned with the earliest attempts to control a disease, usually by a field plant pathologist working against time. It is time for a reexamination (with the light microscope) of the details of leaf surface phenomena in infections under field conditions, prior to more sophisticated work. Modern analysis of the various leaf surface environmental factors is needed, as is a more detailed study of the microbial components which we now know are present on every leaf (Last, 1971). With further work we might discern patterns in the "Achilles heel" pre-penetration biology of fungal diseases. These might well be general patterns or be generalizations associated with particular groups of diseases, or hosts, or parasites, or environmental situations. It seems likely that much is about to be discovered about the leaf surfaces during the early stages of infection. In the most recent issue of the "Annals of Applied Biology", for example, Russell (1976) reports on the significance of mere position on the leaf surface of wheat in the germination of *Puccinia striiformis* uredospores. (The percentage germination was higher on the adaxial surface, particularly on the distal parts, than on the abaxial surface of leaves of adult wheat plants.)

I have been asked to present here some of the particular contributions my research students have made to our picture of leaf pre-penetration biology. In doing so I would like to emphasize that not only is our ignorance immense, but also that whole areas of questioning are neglected here. Interactions between the phyllophane microflora and pathogens are discussed elsewhere (Preece and Dickinson, 1971; Dickinson and Preece, 1976). Light effects on spore germination on leaves need a separate review; present indications are that light may be much more important than hitherto suspected. We are now studying the effects of chemical additives to the leaf surface environment, whether by accident (e.g. pollutants, dusts) or by design (e.g. pesticides, fertilizers). The more obvious (but little studied) animals and their products at the leaf surface (e.g. the microbiological effects of the movements of insects) need study. I omit in this account too, considerations of viruses, bacterial infections and actinomycetes.

For the development of a fungal lesion on a leaf we need a source of spores, and an available leaf. Then follows the external pre-penetration stage. Penetration must occur. There must be internal development of the fungus, followed by release of spores from the lesion. Ultimately we might consider the fate of materials in the lesion (the death of the fungus in the leaf included). Our knowledge of these phases varies. I am concerned in this paper with some aspects of the external pre-penetration stage which includes (1) the arrival of the spore, (2) adhesion to the leaf surface and often (3) external growth prior to penetration. This external growth may (or may not) show each of the common morphologically definable phases of swelling, germ tube production and appressorium formation. We need to focus on where these stages occur on the leaf surface, how long each stage takes to occur, and what environment conditions prevail during each stage. The (apparently) saprophytic microflora (Preece and Dickinson, 1971; Dickinson and Preece, 1976) is part of the microenvironment of the arriving spores. Together with this microflora there may be unexpectedly significant objects —also part of the microenvironment of the pathogen, such as pollen grains (Chou and Preece, 1968).

III. The Arrival of Spores on Leaves

The numbers of airborne spores of particular fungi near a leaf out-of-doors is astoundingly variable with time, as the quantitative measurement of air spora using the Hirst (1952) spore trap reveals. Meredith (1966) noted that airborne conidia of *Helminthosporium* did not exceed three per cubic metre above affected plants, whereas Shanamuganathan and Arulpragasam (1966) working in tea fields, found concentrations of 10 000 basidiospores of *Exobassidium* per cubic metre above bushes affected by blister blight. Hirst (1953) reported the first quantitative records of diurnal patterns of spores in the air, in this finding very marked differences between fungal species. There may be, for example, distinct "wet" and "dry" period situations. Spores of *Erysiphe*, *Alternaria* and *Cladosporium* are dry air spores; Ascospores, such as those of *Venturia* and *Ophiobolus* are constituents of the damp air spora. The ascospores of *Mycosphaerella melonis* are found in highest concentration inside glasshouses when it rains outside (Fletcher and Preece, 1966).

The processes of change in concentration of spores near leaves are complex and little understood. Gregory (1961) gives much information and considers problems of movement in the air and deposition on to leaves by sedimentation and impaction. Turbulence is very important—it is however possible for sedimentation to occur in a moving air stream if it is non-turbulent (Chamberlain, 1967). As a non-turbulent air mass moves over the crop, particles such as rust spores sediment down to the laminar boundary layer of still air surrounding all objects such as leaves. Some spores will then penetrate the boundary layer—others float away from the leaf. Impaction is more complex. Efficiency of arrival at the leaf surface falls off with decreasing spore size, and increases with reduction in width of leaf. Efficiency of impaction is low on dry leaves. "Collection" of spores on natural surfaces is better, for example, than on sticky slides or tape. Rishbeth (1959) found maximum arrival rates of 20 spores of *Fomes* per 100 cm^2 of tree stump surface per hour, and much more commonly recorded 1–5 spores/100 cm^2 per hour deposited quite near sporophores. Barnes (1969) at Leeds compared the number of conidia of *Erysiphe polygoni* arriving on the surface of red clover leaves after exposure near a source of spores in 24 h periods with the atmospheric concentration of spores near the leaves recorded by a Hirst spore trap. These "airborne" and "deposited on leaves" counts are not related directly and there is much to be investigated. Numbers of *Erysiphe* conidia, deposited on clover leaves were often low when trap catches were high. The maximum daily count recorded during a twelve-month study was of 56 per leaf; many daily values being between 0 and 10 per leaf. Relatively high counts of powdery mildew conidia on leaves coinciding with low trap catches were a notable feature of the observations by Barnes. In the case of already infected plants, the number of spores trapped by leaves is directly related to the number of mature sporulating lesions, as was shown in *Exobasidium vexans* infections of tea (Kerr and Rodrigo, 1967). These authors also reported on unexplained greater deposition on more susceptible cultivars. Bock (1962) showed that the final distribution of infecting uredospores of *Hemiliea vastatrix* on coffee leaves can be related to daily rainfall amounts. It becomes clear that each host–parasite–environment situation needs separate study inasfar as arrival of spores on leaves is concerned. It is