The Dynamics of Host Defence

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Preface

When plants are infected by parasites, many changes in cellular metabolism occur. Some of these changes are closely associated with the apparent ability of plant cells to defend themselves. Some changes can be observed by using optical and electron microscopy, whereas others can be detected by extraction of counter-infective substances or by a failure of attempted re-inoculation. This book reviews our knowledge of these changes, the evidence for their involvement in dynamic defence and how these changes are regulated by both parasite and host. Possible approaches to new methods of disease control through manipulation of natural systems are indicated.

Scientific studies and possible applications of dynamic defence systems in plants are of wide appeal. For this reason, the subject was selected as the theme for a symposium for the 4th International Congress of Plant Pathology in Melbourne, Australia, in 1983. Leading international authorities were invited to present reviews of different aspects of the subject. The opportunity to publish occurred at an early stage in planning the symposium so the authors expanded their reviews into the fully documented and detailed chapters of this book.

The book is intended for research workers, university teachers and advanced students in plant pathology, botany and plant biochemistry.

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JOHN A. BAILEY

I. Introduction

Susceptibility and resistance to disease comprise a series of many integrated processes which are activated or have their activity increased by the presence of a pathogen, and which result in its development, exclusion, inhibition or elimination. Interest in these processes probably began after the studies of de Bary (1886) who showed that pathogens were capable of producing enzymes capable of destroying plant tissues and Ward (1902) who indicated that resistance was associated with modifications to the host's metabolism. Since these papers appeared much information has been obtained and this has been discussed and reviewed with increasing regularity. Many of these reviews will be referred to in the subsequent text.

An understanding, and hence possible regulation of host-pathogen interactions represents a major challenge to biologists, geneticists, chemists, biochemists and molecular biologists. The amount of published data is extensive, and the array of different interpretations of this information and consequential concepts are bewildering. As a result, although the main aim of this Chapter is to set the scene for the subsequent contributions by considering the fundamental bases of host-pathogen relationships, it is unavoidable that some personal speculations are also included. It is hoped, that this will both initiate new interests in the ways in which plants succumb to or resist disease, and also act as a stimulus to much needed further experimentation.

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Why study host-pathogen interactions? In brief we do so to provide information: for plant breeders, information on which to base their selections; for agronomists, to provide guidance in agricultural practices; for epidemiologists, to supply a foundation for forecasting models and for crop protectionists, so that any inherent trends of crops towards resistance can be encouraged and integrated into disease control methods. In addition, we hope to identify phenomena which might be exploited as sites for novel protective chemicals, to provide systems for physiologists and molecular biologists to study the regulation of cellular metabolism and finally to satisfy the desire for new knowledge, whose value may only be determined in retrospect.

II. Susceptibility and Resistance

Susceptibility is the justification for all plant pathology, yet in recent years the majority of physiological research has concentrated on the mechanisms by which plants act to restrict the establishment, growth and associated detrimental effects of a pathogen. This Introduction and subsequent contributions reflect such trends, by emphasizing how pathogens may fail to breach walls or wall appositions, how they may be restricted by phytoalexins, or may induce resistance to subsequent pathogens. However, when considering the dynamics of host responses, phenomena associated with susceptibility should receive equal attention to that paid to those associated with resistance. Only by comparing the successful and unsuccessful pathogen and determining the events which dictate the nature of the initial interaction and the ultimate response, i.e. the symptom, will a complete understanding of how plants regulate their response to pathogens, and *vice versa*, be obtained.

Susceptibility is a term frequently used to describe interactions which lead to loss of yield, whilst resistance is used to denote interactions which fail to cause such losses. A definition of resistance based on yield may, however, include situations where a pathogen can infect and grow in tissues, produce a lesion and may even sporulate, but not affect productivity. Clearly such a crop could be considered resistant to disease, yet individual plants would be susceptible to infection. Conversely, if small restricted lesions occur on a particular part of a plant, e.g. flower or fruit, the product of the plant may be unsaleable and hence the crop would be considered highly susceptible.

These important reservations regarding the use of the terms susceptibility and resistance can be further illustrated by discussion of the assignment of "infection types" to cereal-rust interactions (Stakman and Harrar, 1957). Infections of wheat cultivars by *Puccinia graminis* produce a range of symptoms from immunity, which indicates no macroscopic symptoms, but which may include the presence of scattered dead cells, to complete susceptibility which indicates the presence of large sporulating pustules. The gradation between these extremes is represented by a numerical scale 0 to 4 (Table 1). For the purpose of genetics, plant breeding and agronomy, categories 0, 0;, 1 and 2 are considered to represent resistance and categories 3 and 4 susceptibility. However, in categories 1 and 2, sufficient fungus develops in the cereal leaf to allow some spores to be produced.

TABLE 1. Infection Types Produced by *Puccinia graminis* on Wheat (after Stakman and Harrar, 1957)

Infection type	Definition
0	Immune: No rust pustules, no macroscopic symptoms.
0;	Immune: No rust pustules, occasional small flecks of dead tissue.
1	Very resistant: Extremely small rust pustules surrounded by dead tissues.
2	Moderately resistant: Small to medium pustules with band of dead tissues.
3	Moderately susceptible; medium sized pustules with no dead tissues.
4	Very susceptible; large pustules, with no dead tissues.
5	Mesothetic; variable symptoms, often including all the above types on one leaf.

Similar problems arise when comparing infections involving pathogens which produce toxins (Durbin, 1981; Wheeler, 1981). When maize with Texas male sterile (Tms) cytoplasm is inoculated with *Helminthosporium maydis*, race T or race O, visible necrotic lesions are produced. Does this indicate equal susceptibility? On subsequent incubation only

race T produces extensive chlorotic symptoms and only this race causes the great losses of yield associated with the blight disease. As a result, Tms maize is generally considered to be susceptible to race T and resistant to race O. Such a view clearly obscures the initial success of race O, which in other circumstances might be considered as causing a leaf-spot disease.

Thus resistance and susceptibility are ill-defined, comparative terms which do not necessarily indicate two alternatives (cf. recognition) but are often two different consequences of a plant's response to attack (Heitefuss, 1982). Their use may be made more relevant to mechanistic studies if constrained by reference to infection or disease. On balance, however, it is better to try to avoid these terms as much as possible when discussing the details of processes which regulate host-parasite interactions.

Mechanisms of Susceptibility

Most early attempts to understand the sequence of processes in symptom development, from the initial contact between a pathogen and its host to completion of the syndrome were founded on the nutritional basis of pathogen growth (Gaumann, 1950). Fungi, bacteria and viruses are heterotrophic, i.e. they need to obtain their nutrition from pre-existing sources and cannot convert carbon dioxide into organic compounds. They may be saprophytic, deriving their nutrition from non-living materials, or symbiotic, deriving nutrition directly from living organisms. The definition of symbiosis has been much discussed, but an early and still useful view divided it into mutualistic symbiosis, which includes nitrogen fixing bacteria and mycorrhizae, antagonistic symbiosis, i.e. pathogenesis, and neutral symbiosis (Gaumann, 1950). The divisions between these categories are not always easy to assess, as the balance between host and micro-organism may change. They are, nevertheless, useful categories for they draw attention to possible similarities between mutualistic symbiosis, parasitism and pathogenesis, especially when considering modes of infection and establishment of micro-organisms in plant tissues.

The nutritional base of pathogenic micro-organisms can be described as necrotrophic, when they feed on dead tissues, and biotrophic when they feed on living cells (a process which is universal in mutualistic symbiosis). Restricting necrotrophic and biotrophic to describe types of nutrition is better than using them to refer to types of fungi, because

many pathogens can combine both modes of nutrition during a single infection. These organisms, usually termed hemibiotrophic or facultative biotrophic initially grow biotrophically, but this stage is transient and is followed by extensive necrotrophic growth. Many concepts pertaining to host-pathogen interactions have been developed from investigations with hemibiotrophic fungi, e.g. species of *Phytophthora, Colletotrichum, Cladosporium, Venturia,* etc.

In recent years, it has become increasingly evident that susceptibility is not only a matter of appropriate nutrition, but is determined by a network of complex processes which are often regulated by the host (Heath, 1974; Staples and Macko, 1980). Rust diseases provide excellent illustrations of this. Thus, a rust spore must germinate and adhere to its host surface and the germ-tube must grow in the appropriate direction and form an appressorium over a stoma. Subsequent ingress into the tissues requires further directional growth of the emerging infection peg, its adherence to the host mesophyll cell and the production of a specialised haustorial mother-cell. The subsequent cellular interactions of host and pathogen involve synthesis of new host plasmalemma and re-organisation of host metabolism (Scott, 1972). If successful, these processes will lead to functional intracellular biotrophic haustoria and growth of intercellular hyphae. Eventual production of a pustule may be equally complex. Other pathogens may appear less specialized, but successful pathogenesis will often require formation of specialized infection structures and production of appropriate enzymes and/or toxins. Many of these processes are now considered likely to be specifically regulated, e.g. by chemicals on the surface of, or in plant cells or tissues, rather than simply the random consequences of pathogen growth (Staples and Macko, 1980; Kuhn and Hargreaves, 1983).

It is certainly valid that, as well as asking why a plant does not suffer from a particular disease, we should question what are the positive contributions which a plant makes to the establishment of a pathogenic relationship.

Mechanisms of Resistance

Any host-pathogen interaction which is sub-optimal with regard to the pathogen involves a degree of resistance. This may occur at any stage of the interaction, e.g. on the plant surface, during initial infection, during the development of the pathogen or during the development of symptoms. The most commonly considered active responses are the formation of

papillae or cell wall appositions, encapsulation of infection hyphae, and major disturbances to the metabolism of infected tissues, often involving death of cells and production of secondary metabolites e.g. oxidised phenolics, phytoalexins and lignin. Papillae and encapsulations will be discussed in detail by Professor Aist. Further discussion here will be limited to cellular interactions involving localized cell death and accumulation of phytoalexins.

Localized Cell Death. In certain plant species or cultivars, many pathogens will establish biotrophic infection hyphae, whilst in others the initially infected cell(s) die quickly, often becoming darkly pigmented, and further growth of the pathogen is prevented. The occurrence of localized pigmented cells is often referred to as evidence of a hypersensitive response. Ideally, perhaps, hypersensitivity should be restricted to interactions invoking immediate death of the initially infected cell, but it is often used to describe symptoms which vary from single dead cells, to small groups of cells or even to small limited lesions. As such, hypersensitivity is a common response of plants to fungi, bacteria, viruses and nematodes (Muller, 1959; Kaplan and Keen, 1981; Bushnell, 1982; also Chapter by Van Loon).

Hypersensitivity is an association of dead plant cells with the failure of a pathogen to develop. It does not imply any mechanistic explanation and there has been much debate as to the temporal relationships between death of affected plant cells and death or inhibition of the pathogen. For many years it was assumed that plant cell death or its consequences were the cause of fungal growth inhibition. But doubts as to the validity of the traditional view were expressed in 1972, by Kiraly, Barna and Ersek who showed that when biotrophic infection hyphae of *Phytophthora infestans* were killed by exogenously applied antimetabolites, the infected cell died soon afterwards, accompanied by the production of phytoalexins. A similar progress of events occurs in *P. megasperma*-infected soybean hypocotyls after treatment with the fungicide metalaxyl (Ward *et al.*, 1980). It was suggested that processes similar to those induced above, i.e. the death of the pathogen leading to death of infected cells, occurs in natural hypersensitivity (Kiraly, Barna and Ersek, 1972). This alternative view proved to be a great stimulus for research by other workers, and detailed investigations of several host-pathogen hypersensitive responses, e.g. Bremia-lettuce (Ingram *et al.*, 1976), Colletotrichum-bean (Bailey, 1982a), Phytophthora-potato

(Tomiyama, 1982) and Puccinia-oat (Mayama *et al.*, 1982) have now been reported.

A major problem in interpreting hypersensitive responses is understanding and defining cell death (= necrosis). Necrosis, rather than being taken to signify only the death of a cell(s), is more usually used to refer to cells which are both dead and darkly pigmented. This tends to confuse an initial determining event, e.g. membrane damage, with a final symptom, which may occur several days later. A cell may be dead for several hours before dark pigmentation is visible. When these points are considered, it is clear from the host-pathogen interactions referred to above that infected cells show indications of damage many hours before growth of the pathogen is affected. Furthermore, hypersensitivity should not be viewed as involving death of the pathogen until this has been proved in each specific interaction. Hyphae of *C. lindemuthianum* remained viable within hypersensitive cells for several days (Bailey and Rowell, 1980). Perhaps ironically therefore, the traditional view of hypersensitivity, i.e. that the infected cell dies first, is now well established.

The importance of localized plant cell death is also consistent with the complex interactions which occur between the dead cell and the surrounding healthy tissues. Of paramount importance appear to be those involved in the synthesis and accumulation of phytoalexins, which are now considered an integral part of hypersensitive responses.

Phytoalexins. Phytoalexins are antimicrobial secondary metabolites which form part of a greater activation of new plant metabolism, which occurs as a consequence of stress, particularly following microbial infections and after treatment with toxic compounds or toxic microbial metabolites. Investigations into their nature, metabolism and mode of action, their modes of synthesis and the mechanisms involved in activating synthesis have predominated recent studies of active defence (Bell, 1981; Bailey and Mansfield, 1982; Keen, 1982b). The relevance of phytoalexins to resistance mechanisms can be summarized as follows. They accumulate in infected plants, at the site of the host-pathogen interaction where tissues are killed. They may attain concentrations which would prevent pathogen growth *in vitro* at the time when growth inhibition can be first detected. On this basis phytoalexins can be an important determinant of inhibition of pathogens in hypersensitive responses and during limitation of lesions (Mansfield, 1982).

The mechanisms involved in the synthesis and induction of synthesis of phytoalexins present intriguing problems which are now attracting the attention of some of the world's leading plant biochemists. Much evidence is appearing which indicates that production of phytoalexins requires the integrated *de novo* synthesis of a series of enzymes, e.g. phenylalanine ammonia lyase and chalcone synthase, required for the formation of isoflavonoid phytoalexins (Lawton *et al.*, 1983). The mechanisms which induce the synthesis of these enzymes and their specific messenger ribonucleic acids is more problemmatical. However, in the last few years, there have been indications that these syntheses are mediated by metabolites of the host (constitutive or endogenous elicitors), which are released as a consequence of infection or treatment with various chemicals (Bailey, 1982b; Albersheim *et al.*, 1981). The release of the plant elicitors may be a direct consequence of cell damage (Hargreaves and Bailey, 1978) or may require the additional action of host enzymes (Lyon and Albersheim, 1982), which may also be activated or released as a consequence of cell injury. Either of these explanations would be consistent with present knowledge of hypersensitivity. Alternatively, enzymes produced by the pathogen may be the effective elicitors. Thus an endopolygalacturonase from *Rhizopus stolonifer* is an elicitor of casbene synthase, the enzyme responsible for the formation of casbene, a phytoalexin from *Ricinus communis* (Bruce and West, 1982).

At this time, emphasis is moving from metabolites of the pathogen to those of the host. No clear picture has emerged, although cell injury or death appear to be vital events. Further progress will depend on researchers distinguishing between microbial elicitors which act by damaging plant cells and elicitors from plants which are not toxic and which may directly activate *de novo* enzyme synthesis.

In summary, the important modern concepts concerning active defence based on phytoalexins include the following:

1. Phytoalexins are low molecular weight secondary metabolites, produced in response to localized death of plant cells. They are not produced during biotrophic infections.
2. Synthesis of phytoalexins occurs in adjacent healthy cells in response to materials diffusing from the damaged cells.
3. Synthesis of phytoalexins is a consequence of *de novo* synthesis of many enzymes.

4. Phytoalexins accumulate in both resistant and susceptible necrotic tissues. Resistance occurs when the concentration of phytoalexins are sufficient to restrict pathogen development.
5. Accumulation of phytoalexins is a process remote from the recognition events which initially distinguish host-pathogen interactions.

III. Species and Cultivar Specificity

Plants, like animals, have been continually exposed to foreign organisms and have evolved mechanisms enabling them to support or overcome the activities of these agents. Evolutionary pressure may have worked in different directions, for it is advantageous to plants to be receptive to beneficial organisms, e.g. nitrogen fixing bacteria and mycorrhizae, but to be resistant to damaging ones.

The consequences of evolution which we observe to-day are species and cultivar specificity. Thus a given pathogen (or symbiont) usually grows in a very limited range of plants and often in only a single species. Equally a single plant species will be susceptible to only one or, at most, a few pathogens. Hence, although many pathogens may be present in its immediate environment, a plant can remain healthy. In reality, many plants from both wild and crop species, suffer from disease at some time during their life, especially those growing in warmer climates. Examples of species specificity are evident to all plant pathologists. Thus elm trees succumb to a wilt disease caused by *Ceratocystis ulmi* so readily that this tree has virtually disappeared from the eastern regions of North America and from most of southern Britain. This pathogen has negligible effects, however, on potatoes. Conversely, the equally devastating pathogen *Phytophthora infestans* destroys potato plants, and in the years 1845 - 1850 was the direct cause of more than 1 million deaths in Ireland. It is incapable of damaging elms. These examples of specificity are extreme. It should, however, be realized that host specificity is also evident between quite closely related species of pathogen and host. For example, French bean (*Phaseolus vulgaris*) is susceptible to its rust *Uromyces phaseoli* but not to *U. viciae* or *U. vignae* which are pathogens of the related legumes *Vicia faba* and *Vigna sinensis* respectively. The resistance of species is now commonly referred to as non-host resistance and has been the subject of much recent interest (Heath, 1980).

In addition to, and possibly superimposed upon species specificity is cultivar specificity. This exists in many of our crop plants due to the selection, often after an extensive breeding programme, of new types (cultivars) of a species which resist an organism normally considered capable of attacking that species. Resistance in cultivars is often referred to as host resistance, to compare directly with non-host resistance.

As indicated earlier, specific interactions do not only involve potential pathogens. Nitrogen fixing bacteria *Rhizobia* are specific to the Leguminosae and different species of *Rhizobia* specifically infect and nodulate different legumes.

Finally there remain some micro-organisms which do not show a high degree of specificity. For example, *Plasmodiophora brassicae* will affect many species of Cruciferae, whilst *Agrobacterium tumefaciens* affects most dicotyledonous plants, and ectotrophic mycorrhizae affect both dicotyledons and monocotyledons.

Gene for Gene Hypothesis

Environmental factors may greatly influence the expression of symptoms, but the fundamental nature of interactions between a host cultivar and a pathogen are determined by their respective and often interactive genotypes. The selection and breeding of new disease resistant cultivars has led to the identification, and also in many cases to the existence of new forms (physiological races) of a pathogen which can overcome this resistance. As a consequence, many cultivars of the major annual crops now exist and they are exposed to attack by many races of a pathogen. The genetical relationships between cultivars and races was first studied in detail by Flor (1956). The subsequent amount of literature and the success of plant breeding indicate the very great contribution of Flor's gene for gene hypothesis to many aspects of plant pathology, including attempts to understand the molecular basis of cultivar susceptibility and resistance (Day, 1976; Ellingboe, 1981). The study of the genetics of cultivars of flax and of isolates of the rust pathogen *Melampsora lini* enabled Flor to conclude that a close relationship exists between the genes of a cultivar and those of the pathogen races, such that for each gene determining resistance there is a specific gene in the rust fungus which conditions the ability to overcome resistance. Thus, for cultivars