PHYSIOLOGY AND PLANT PATHOLOGY*

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ABSTRACT

The impact of physical sciences, particularly biochemistry, has played a significant role in understanding etiology and syndrome in pathogenesis. Critical tissue respiration and enzyme changes, deranged carbohydrate and nitrogen metabolism, transpiratory disturbances and ionic imbalance produced by fungal toxins, exaggerated auxin relationships, formation of abnormal metabolite (s) (phytoalexins) have all contributed to a better understanding of the 'sick' plant.

Plant virologists have made phenomenal progress in the applied field of the biochemistry of the infected plant and some of the recent researches on the nature of viruses and control measures adopted are worth emulating in other fields of plant pathology.

A new field is developing round environment and disease proneness. This has reference to the rice blast disease where low nyctotemperatures for a long enough period makes for alterations in the nitrogen metabolism of the host and is governed by the balance between primary nitrogen metabolism and secondary metabolic events leading to synthesis of structural metabolites.

INTRODUCTION

MUCH of the symptomatological approach to problems of plant disease has given way in recent years to an integrated study of cause and effect. It is becoming increasingly clear that adequate knowledge of plant physiology and biochemistry is a prerequisite for interpreting metabolic changes

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in plants under pathogenesis. Pioneering work in this area is from plant virologists. They had the advantage of dealing with an obligate parasite producing a systemic disease where the metabolism of the healthy plants suffered a crippling blow. In fact, the initial novelty of studying one or two aspects of 'stress physiology' has now yielded to a multi-sided approach to understanding the many metabolic changes that go to make the subject fascinating (Goodman *et al.*, 1967).

Respiration and Enzymic Changes

One of the very first attempts was to measure plant respiration as an index of disease and, in fact, an increased respiration in tissues is known to accompany pathological conditions following infection by bacteria, fungi and viruses (Allen, 1953, 1954; Uritani and Akazawa, 1959). Alterations in respiratory pattern became apparent long before dechlorophyllation, abscission of leaves, necrosis, changes in leaf morphology or wilting were discernible.

The merit of Allen's pioneering work lay in showing that enhancement of respiration in mildewed wheat leaves may be partly due to the abolition of the *Pasteur*-effect by some toxin uncoupling respiration from the energy-yielding mechanisms of the cell. Similar evidence was presented later by Daly and Sayre (1957) for derangement of the *Pasteur*-effect, but they explained it differently. Increased respiration under pathogenesis was according to them strongly resistant to malonate indicating that a major portion of the electron transfer was conducted not *via* the succinic oxidase-cytochrome system, but through a new respiratory pathway in diseased plants which bypasses the normal Krebs cycle. A striking fact that emerges from a study of the effect of toxins on respiration is the rather widespread early occurrence of respiratory increases resulting from uncoupling of respiration from energy-requiring processes through action on oxidative phosphorylations. Consequent on the failure of a regulatory mechanism the host reserves and their intermediates become available to the parasite. Furthermore, there is an inhibitory effect upon respiration and respiratory enzymes of toxins which act against microorganisms. From the enzymological point of view in host-parasite relations the emphasis is on oxidative enzymes. This is so because the pathway of oxidative processes in healthy tissues is well known and the application of this knowledge on diseased tissues is now meaningfully interpreted. Taking the case of *Ceratostomella fimbriata* infection of sweet potatoes, respiratory increase could be induced artificially by adding a toxin isolated from

infected tissues (Akazawa, 1956; Akazawa and Uritani, 1955). The mechanism of stimulation was an uncoupling as a consequence of stimulated ATP-ase activity. Hand in hand with increased ATP-ase activity in the healthy tissues and surrounding diseased tissues stimulation of protein and organic phosphate synthesis has been recorded. The acceleration of energy-consuming processes has the same consequences as enhanced ATP-ase activity. In fact, both processes may lead to a higher level of phosphate acceptors (ADP) which are known to regulate respiratory rate. Other enzyme systems are also known to be activated in this series of oxidative changes such as cytochrome oxidase, peroxidase and polyphenol oxidase. Thus, the enzymatic machinery of diseased cells gives rise to chemical entities which by upsetting the enzymatic reactions of the parasite may impede its spread and development *in vivo*. In other words, it may be regarded as a parasite-induced chemical resistance.

Deranged Growth Regulatory Mechanisms

Allen's postulate of a toxin-induced change in tissue respiration was later examined from the point of view of auxin levels *in vivo* in safflower under rust infection (Daly and Inman, 1958). They showed correlation between development of hypocotyls and the presence of indole compounds. The metabolic activity of rust-affected safflower was thus a consequence of a primary disturbance of hormonal balance during the initial stages of infection. The increased growth that occurred during the mycelial development of the parasite could have arisen from removal of a growth inhibitor or by increasing growth-stimulating substances in invaded tissue. Later workers showed in a more precise manner increase in indole acetic acid equivalents in tomato tissue invaded by *Verticillium albo-atrum* (Pegg and Selman, 1959). Indeed, growth substances have been found in tissues infected with bacteria, fungi, viruses and nematodes. Although a specific role for auxin in most wilt diseases has not been established, tomato plants infected by the bacterium *Pseudomonas solanacearum* has been shown to exhibit leaf epinasty and adventitious root formation (Sequeira, 1963). Increased levels of indole-3-acetic acid (IAA), an additional auxin and a growth inhibitor have been shown in *P. solanacearum* infected tobacco and banana tissues. There is, however, one practical difficulty. The biosynthesis of IAA proceeds along similar lines in host and pathogen and, therefore, determining the source of IAA in diseased tissues with any degree of certainty has posed an experimental problem. Inhibition of IAA-oxidase in wound sites has been demonstrated. However, auxin destruction alone cannot explain growth changes and auxin levels

between diseased and healthy tissues. It should be mentioned that mechanism of auxin action brings in its trail plasticity of the cell-wall and consequent effect on pectic substances, increase in a number of oxidative enzymes and in accumulation of phenolic substances. It has even been suggested that IAA could interfere with normal lignification of host tissue.

Toxins and Water Relations of Plant Tissues

Phytopathologists have been concerned for many years with the mechanism of pathogenic fungal wilts and the causes that lead to acute water shortage in such tissues (Gäumann *et al.*, 1952; Gäumann, 1958; Dimond and Waggoner, 1953; Sadasivan, 1961; Subramanian and Saraswathi-Devi, 1959). The consensus of opinion is that many of the fungal toxins produce permeability changes depending on the extent of injury to cells. Fusaric acid, alternaric acid, penicillic acid, patulin, streptomycin and many others have been shown to impair permeability even at low concentrations. Thus, changes in osmotic pressure result from variations in water content due to continued loss of water through transpiration. In some cases an ionic imbalance in the leaf cells have been recorded both by conductivity studies (Gnanam, 1956) and by analysis of leaf ash samples (Sadasivan and Kalyanasundaram, 1956; Sadasivan and Saraswathi-Devi, 1957). The mechanism of this exaggerated water loss during the onset of pathogenesis has been only partially unravelled; nevertheless, its fundamental nature as a problem of plant physiology remains alive. For instance, how far is the chain of events reversible and how far can the ionic imbalance and excessive loss of one element or the other be made good *in vivo*? These and related questions on the loss in integrity of the cell-wall, the plasmalemma, the protoplast and the tonoplast will have to be answered by careful experimentation.

Environment and Disease

Recent contributions on the blast disease of rice by *Pyricularia oryzae* from this laboratory have opened up new areas of research (Suryanarayanan, 1958 *a, b*; Sadasivan *et al.*, 1965; Ramakrishnan, 1966; Suryanarayanan, 1967). Nitrogen metabolism of the rice plants exposed to low night temperatures for a period of a fortnight or so has been shown to increase their proneness to blast. Discussing plant resistance to a number of facultative parasites (such as *Drechslera oryzae, Pyricularia oryzae, Cercospora personata, Fusarium moniliforme* and *Fusarium vasinfectum*), Sadasivan (1968) has indicated a role for aromatic metabolites like phenylalanine

in the resistance of rice varieties to *D. oryzae* and *P. oryzae*. It has been further shown that genotype-nyctotemperature interaction associated with resistance to blast affects primarily the nitrogen metabolism of the host. Low nitrate reductase activity and high nitrate accumulation are characteristic of rice plants exhibiting resistance to blast. Broadly speaking, resistance to facultative parasites could be visualized as governed by the balance between the primary nitrogen metabolism and secondary metabolic events leading to synthesis of structural metabolites. In future studies in this area, the emphasis, therefore, has to be on understanding the biosynthetic rates of aliphatic and aromatic amino acids, synthesis of isoenzymes and other proteins as well as on the formation of resistance factors like phytoalexins *in vivo* under highly controlled nyctotemperature regimes. This whole concept of resistance to blast being "polygenic", particularly as genotype-nyctotemperature interactions are decisive in the expression of resistance, stems from the important work of Suryanarayanan and is summarized in his recent papers (Suryanarayanan, 1966, 1968).

Advancing Frontiers in Plant Virus Research

(*a*) *Fundamental Studies on Virus Particles.*—We shall now turn our attention to the physiology of virus-infected plants. Virologists have made spectacular progress on deciphering the intricate problems connected with structure of viruses. That is probably best left outside the scope of this lecture except to state in a passing way that the difficult tasks of working out the nucleotides of the viral nucleic acid and the amino acid sequences of the viral protein have been successfully accomplished. The peculiar behaviour of the satellite virus (which is the smallest known virus and has a relationship with tobacco necrosis virus for *in vivo* multiplication) in not being able to spare nucleotides for infectivity after coding its small number of nucleotides for its structural protein is in itself a most significant discovery (Bawden, 1966). Virus multiplication is currently thought of as a derangement of the nucleic acid metabolism of the host cell with secondary effects on protein metabolism. The most fascinating problem is the 'disrobing' site in the cell of the infecting virion where it releases its nucleic acid. Following this polymerization of the nucleotides to duplicate the virus and the subsequent coding of the nucleic acid for its structural protein units are topical questions. The likely 'enrobing' site is considered to be the nucleus and the site for protein synthesis the ribosomes. The discovery of an extremely small-sized virus particle which is a free nucleic acid (a 'naked' virus) with a double helical structure and

with at least one strand of it composed of RNA (potato spindle tuber virus) capable of being transmitted with ease and having remarkable stability may lead to a re-evaluation of the importance of the viral protein coat for infectivity (Diener and Raymer, 1967).

(*b*) *Virus-free Zones and Chemotherapy.*—With the newer knowledge from morphologists and physiologists that meristematic tissues could be cultured *in vitro* the virologist has succeeded in tissue-culturing apical meristems of virus infected plants and showed that virus-free clones could be got from them. This indicated that the meristematic region was virus-free (Kassanis, 1965). Alongside these advances much fundamental work has gone on in the field of chemotherapy. The vital part of a virus particle is its nucleic acid and any chemical that could interfere with the nucleic acid metabolism could be employed in inactivating the virus *in vivo*. The principle involved was that of substituting a group in such a way as to make the chemical still usable in metabolism of the plant but in a less effective manner. Analogues of purine and pyrimidine bases which are known to make up the nucleic acid were used with some limited amount of success. For example, thiouracil when used seemed to prevent the entry of uracil into the nucleic acid of the virus thus forestalling formation of the virus particle. It has also been explained as blocking the participation of uracil in viral multiplication or by disorganizing the metabolism of the plant.

(*c*) *Virus-Vector Complexity.*—Any treatment of viruses would be incomplete if the complexity of virus-vector relationship *vis-a-vis* host infectivity is not dealt with. The discovery that plant viruses could be detected by electron microscopy in the vectors is interesting in itself. Even more interesting is the discovery that the wound tumor virus has inefficient and efficient hopper vectors in *Agalliopsis novella* and *Agallia constricta* respectively and that the virus was systemically distributed in the efficient vector in the mid-gut, musculature, blood, fat bodies, trachaeoblasts and ventral ganglia, thus making it the first evidence of plant viruses invading nervous tissues of insect vectors (Granados *et al.*, 1967).

(*d*) *Virus Infections and Host Physiology.*—There are many other problems connected with the physiology of the host and viral infections that make the study of this subject of extreme interest. For instance, study of the accumulation of ions, reduction in phosphorus, increased or decreased respiration, auxin imbalance, oxidase systems, changes in carbohydrate synthesis, amino acid contents, amides and a whole host of metabolites would be rewarding. These studies could be conducted in diffe-

rent genetic varieties of plants that show localization of viruses and also differing systemic symptoms in the presence of viral strains. They could also be extended to areas where chemical inhibitors for viral synthesis are used. Environmental conditions which affect the type of viral particles synthesized *in vivo* is another area of research (Kassanis and McCarthy, 1967). These workers have shown that a new strain of Dolichos enation mosaic virus which is a serotype of tobacco mosaic virus infecting leguminous plants produces more defective virus particles than the parent strain when infected plants are kept at 20° C. but not at a temperature above 32° C. but both strains multiplied faster at 32° C. than at 20° C.

The Future

There appears, therefore, no end to spotting problems of interest in a research field like this and the more we inject into this area fundamental concepts of plant physiology and biochemistry the richer will be the dividends we could expect in the applied science of phytopathology. A whole array of chemicals have been evolved for plant protection and many have to be carefully screened for phytotoxicity. This in itself is a major problem. Unless redoubled efforts are made in understanding the physiological processes of plant life in different environments and under differring etiological conditions, much of what remedial measures we prescribe for plant protection would remain empirical.

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