ON BETTER UNDERSTANDING OF PLANT PATHOGENS*

By T. S. Sadasivan, F.A.Sc.

(Centre for Advanced Studies in Botany, University of Madras, Madras-5)

Received June, 1971

Plant pathology has benefited by the application of basic knowledge from other disciplines. This has enabled the subject to grow unhampered. Some of the recent trends are discussed here.

I. Rhizosphere Microfloras and Disease Expression

(i) *General concepts.*—That plant roots exude a wide variety of substances utilizable by micro-organisms of the rhizosphere (microflora of the root-soil interface is defined as the rhizosphere) or rhizoplane (rhizoplane includes external surfaces of plant roots and closely adhering soil or debris) is now a proven fact. Nevertheless, all exudates are not beneficial to microbial growth and proliferation in the rhizosphere. In fact, we have situations where negative rhizosphere effects have been noticed as in the case of *Brassica juncea* and *Allium cepa.* In these plants, in addition to root exudation of amino-acids and sugars, a number of organic acids such as citric, malic, oxalic and tartaric were identified and *in vitro* these acids inhibited microbial growth and multiplication. There are also instances where cultivars of the same host species susceptible to soil-borne fungal diseases showed a greater rhizosphere effect, both qualitatively and quantitatively, than resistant cultivars. Genetic varieties of crop plants bred for resistance are also known to exude through the roots metabolic depressants that inhibit spore germination of pathogens.

Evidences for the production of antibiotics in soils *in situ* are also accumulating. However, the functioning of antibiosis in soils as a deterrent to the rise and fall of pathogenic forms in the rhizosphere or rhizoplane has remained speculative. Many fungal propagules from soils do not germinate

---

* Part of the material forming subject matter of the National Lectures of the University Grants Commission, 1970.

Memoir No. 128 from the Centre for Advanced Study in Botany, University of Madras.

readily *in vitro* and indirect reasoning has led to the supposition that a fungistatic factor inhibiting spore germination was operative. Also, fungal spores introduced into fresh fertile soils are unable to germinate due to this fungistatic factor but such spores germinate readily when susceptible seedling roots grow in their immediate vicinity.

(ii) *Exudates from germinating seeds.*—Our interest in exudates from germinating seeds stemmed from the observation that rice seeds of varieties susceptible to the foot-rot fungus *Fusarium moniliforme* escaped disease when pre-germinated seeds were raised as nursery in infested soil. It was argued that energy substances may have exuded into the rhizosphere/rhizoplane and acted as stimulants or as a nutritive springboard for spore germination and initiation of the pathogenic phase of *F. moniliforme*. *Oryza sativa* var. MTU 9, highly susceptible, and *O. sativa* var. PTB 1, moderately susceptible to foot-rot, were examined. Exudates from germinating seeds of the highly susceptible MTU 9 revealed that the bulk of the amino-acids and sugars were excreted in the first 72 hr after which hardly traces were demonstrable at 96 hr (Rajagopalan and Bhuvaneswari, 1964). Such pre-germinated seeds when sown in infested soil brought down the mortality rate from 90% (in the case of seeds of the same variety directly germinated in infested soil) to 22%. Therefore, the importance of the age of the seedling, inoculum potential and availability of host exudations during seed germination at the infection court had, during the critical period of the first 72 hr, all the necessary prerequisites for an increased seedling mortality in this disease. We have recently examined critically, as an academic question, exudates from seeds of several strains of *indica* and *japonica* rice with differing disease resisting characters at different stages of germination (Leelavathy, 1970). Leucine, glutamic acid, valine and alanine constituted more than 80% of the total amino-acids exuded. The three pure line indicas under test gave out maximum amounts of amino-acids. More amino-acids and sugars exuded from seed surface sterilized with mercuric chloride than the untreated seeds except in the initial stages. Furthermore, the presence of certain amino-acids in the resting seeds but absent in exudates of germinating seeds and the appearance of new amino-acids in exudates during germination have been recorded in this study.

(iii) *Exudates from growing seedlings.*—Root exudates from two varieties of rice, resistant (GEB 24) and susceptible (MTU 9) to the foot-rot organism, *Fusarium moniliforme*, grown under aseptic conditions have been analysed by us and found to have qualitatively four amino-acids in common (aspartic

and glutamic acids, tryptophan and lysine) but the resistant variety had in addition cystine, asparagine, tyrosine and methionine, the first two in sizable quantities (Andal *et al.*, 1956). The susceptible rice variety examined had quantitatively more amino-acids and sugars exuding but less organic acids compared to the resistant variety. The presence of cystine in the resistant variety is of considerable interest as it has been suggested to be a resistance factor in amphidiploid cottons showing resistance to the wilt pathogen *Fusarium vasinfectum.* Quantitatively, the root exudates of diploid susceptible cottons ($n = 26$) contained greater quantities of amino-acids and vitamins than the resistant amphidiploid plants ($n = 52$). Furthermore, the quantities of amino-acids and vitamins recorded for exudates from the diploid plants were noticed to go down with pathogenesis. In general, influence of cotton roots on amino-acid and vitamin requiring bacteria was more with the former group than the latter (Sulochana, 1962 *a, b, c*).

(iv) *Mycofloras of the rhizosphere/rhizoplane.*—Despite the claim that root-tips of actively growing plants remain uncolonized by fungi during the whole period of active growth we have evidence to show that *Fusarium solani,* an unidentified phycomycete and occasionally *Rhizopus oryzae* were found on root-tips and root-caps of cotton seedling roots. Also, *Alternaria, Aspergillus, Cunninghamella* and *Fusarium* species have been recorded for the root-tips of *Crotalaria juncea* (Sulochana, 1968). Recent intensive studies on the rhizosphere and rhizoplane mycofloras of crop plants like *Vigna sinensis, Pennisetum typhoides* (3 varieties), *Lycopersicon esculentum, Brassica juncea* and *Trichosanthes sanguinea* grown in different soils have yielded interesting results (Natarajan, 1969). The rhizosphere mycofloras of four plant species grown in a given soil showed that no fungal species was exclusively found in the rhizosphere of any one of these plants. However, certain fungal species were numerically more (as they appeared as large number of colonies in the dilution plates) in certain plant species but not in others showing thereby a concentration of the propagules of these particular species in the rhizosphere soil. Comparing the species composition of the rhizosphere mycoflora of the three varieties of *Pennisetum typhoides,* the dominant species of fungi were generally the same for all three varieties. Rhizosphere mycofloras in relation to age of plants showed occurrence of certain species in the early samplings and they continued to be present till fruiting or late senescence.

In contrast to the behaviour of mycofloras in the rhizosphere those examined in the rhizoplane showed that whatever the plant species, the soil in which it was grown, or the age of the plant, the root surface mycoflora

included only a relatively limited number of species. However, the age of the plant had an effect on the species composition of the rhizoplane mycoflora. For instance *Fusarium semitectum* and *F. lateritium* occurred only after flowering in the rhizoplane of *Vigna sinensis* and *Pennisetum typhoides* (Natarajan *loc. cit.*). The most common species in the rhizoplane was *Fusarium solani*. Less common were *F. scirpi*, *F. semitectum* and *F. lateritium*, species of *Rhizoctonia*, *Cylindrocladium* and *Neocosmospora vasinfecta*.

(v) *Foliar spray of nutrilites and chemicals and rhizosphere/rhizoplane mycofloras.*—It was shown by us in 1959 that foliar application of urea to rice seedlings resulted in preferential stimulation of species of *Penicillium* with concurrent reduction in bacteria and actinomycete flora in the rhizosphere (Ramachandra Reddy, 1959 *a*). This indicated that urea sprays brought about an altered rhizosphere environment by exerting a change both in the nature and amount of root exudates. The rise in *Penicillium* population and reduction in number of other micro-organisms could well be a case of competition for the food base (exudates) and/or an antibiosis factor brought in by the increased activity of penicillia. We have recently tried pretreating roots of one-week old rice seedlings with patulin, griseofulvin, gibberellin, actidione, agrimycin and urea and studied the effect of these substances on quality and quantity of rhizosphere microfloras. With 1 ppm and 5 ppm of griseofulvin, gibberellin, actidione, patulin and agrimycin a negative rhizosphere effect was noticed for bacteria and fungi but with agrimycin at 50 ppm, all three groups, actinomycetes, bacteria and fungi were stimulated in the rhizosphere (Ramachandra Reddy, 1968 *a*). With 0·1 M urea treatment, however, no change in the rhizosphere was observed for bacteria and fungi but actinomycetes were stimulated. Apart from these quantitative changes, roots pretreated to some of these substances showed shifts in the percentage of penicillia and aspergilli in the rhizosphere. While penicillia were stimulated by patulin and griseofulvin treatments, actidione stimulated other groups of fungi but reduced numbers of aspergilli (Ramachandra Reddy, 1968 *b*).

Changes in the mycoflora of rice in the rhizosphere, rhizoplane and phyllosphere (leaf surface) consequent upon spraying a fungicide has also been followed. We have used the fungicide Kitazin (O, O-Diethyl-S-benzyl thiophosphate) as a foliar spray on rice var. Co 18. There was a lowering of the number of fungal propagules/gram of dry soil in the rhizosphere of the sprayed plants. In the rhizosphere lesser number of species were isolated from the sprayed plants as compared to controls and in the phyllosphere, where there was direct contact of the sprayed chemical, more inhibition of

fungi than in other areas of the lamina was seen. Taking all three situations, the rhizosphere, rhizoplane and phyllosphere, with increasing concentration of the chemical, there was a diminution in total fungal numbers in the rhizoplane and phyllosphere but not in the rhizosphere. These results indicated that Kitazin was toxic to many saprophytic forms in all three micro-environments in rice (Sullia, 1969).

(vi) *Rhizosphere microfloras in diverse plants and under stress.*—Actinomycete, bacterial and fungal numbers from the rhizosphere of mesophytes were higher than those of aquatic and marshy plants. Furthermore, the occurrence of specific rhizosphere micro-organisms depended on the plant studied rather than the ecological habitat (Chinnayya and Agnihothrudu, 1953). We have also examined some Pteridophyte members belonging to the Equisetaceae, Cyatheaceae and Polypodiaceae. All plants examined showed a positive rhizosphere effect for both bacteria and fungi and quantitatively some had more microbes and some less. Qualitatively, both penicillia and aspergilli occurred freely and, in particular, a species of *Trichoderma* was present in almost every case examined. Pathogenic forms of fungi like the ubiquitous *Fusarium* were rare (Ramachandra Reddy, 1959 *b*).

A somewhat new approach has recently been made. We have been examining rhizosphere microfloras of plants infected with a systemic virus. The choice was the legume *Dolichos lab lab* infected with Dolichos enation mosaic virus. The effect of virus infection was seen not only in the rate of accumulation of organisms in the rhizosphere against time of incubation and progressive virulence of symptoms but also in the manifestation of maximum rhizosphere effect in relation to age of plant (Lakshmi-Kumari, 1964). Moisture content of soil and the seasonal variation in climate affected the rhizosphere effect in infected plants. All these indicated that altered host-parasite interaction under pathogenesis brought in its trail an altered exudation pattern which in turn was reflected by a changing rhizosphere effect.

(vii) *Rhizosphere microfloras and plant disease.*—There are some well-known examples of soil microfloras modifying the parasitic phase of fungal pathogens. *Ophiobolus graminis* is one such where the pathogen penetrated into the roots of many flowering plants grown in sterilized soils while under natural conditions these plants were immune to infection. Similarly, *Fusarium vasinfectum*, the cotton wilt fungus could produce only a slow tempo wilt which took 30 days to develop under unsterile conditions as compared to a quick wilt of 8–10 days in sterile soil substrate. However,

there are contradictory results to the two cited above. A case at issue is the infection of pea plants by *Ascochyta pinodella* where the rhizosphere microflora had little or no influence on pathogenesis between sterilized and unsterilized soils (Bhuvaneswari *et al.*, 1965). We have demonstrated that a sizable actinomycete population antagonistic to the pathogen *Fusarium udum* was present in the rhizosphere of the resistant pigeon pea plant *Cajanus cajan* while the rhizosphere of the susceptible variety was more conducive for the survival of the pathogen. A somewhat similar situation existed in *Oryza sativa* where the healthy plants of the susceptible variety harboured in their rhizosphere greater numbers of physiologically more active groups of bacteria than the resistant variety studied. Among the nutritional groups of bacteria present in the rhizosphere, the susceptible variety had in its rhizosphere a greater percentage of organisms requiring amino-acids than those requiring amino-acids plus growth factors. The resistant variety, on the other hand, appeared to stimulate abundant growth of autotrophic forms. This method of studying the metabolism of infected plants using, as an index, the rhizosphere microfloras and their physiological grouping *vis-a-vis* pathogenesis is an interesting one and capable of being extended to the study of perennial plants (Sadasivan, 1962).

Exudates from roots and leaves and their role in microbial fecundity, be they saprophytes or parasites, have aroused much interest in the microbiologists and the plant pathologists in recent years as they seem to have the key to many complex pathological syndromes that have as yet not been well understood. To many, plant roots have no more meaning than acting as mechanical props to the tops that they see, but to those that are trying to understand the cause and effect among pathogenic fungi the interactions now presented unravel a new starting point for understanding the behaviour of a given genotype in different growth environments and proneness to disease.

II. PHYTOTOXICITY AS A FACTOR IN THERAPY

(i) *Problems in therapy.*—The success of any chemotherapeutant depends on the selective toxicity of the chemical, its ease of absorption and translocation in a plant and most important of all its freedom from causing ill-effects on plant metabolism (Sadasivan, 1966). There has been much rethinking in recent years on the wisdom of using many insecticides and fungicides that may have a prolonged residual effect in plants and soil and, indeed, the once thriving DDT, Aldrin and Dieldrin industries are slumping after reports from scientific communities have incriminated them as interfering

with plant growth and human metabolism. Many pesticide, insecticide and fungicide schedules are now known to alter soil-plant rhizosphere interactions, membrane permeability, translocatory and transpiratory processes, induce changes in oxygen tension of tissues and ion uptake, affect photosynthesis, nucleic acid and protein synthesis. In recent years fungal toxins affecting plant metabolism have been described and antibiotics are known to be formed in soils and plants in the rhizosphere/rhizoplane. These have been shown to be readily translocated to the shoots in a very short time.

(ii) *Toxins and Toxaemia.*—The vivotoxin fusaric acid produced by the cotton wilt fungus *Fusarium vasinfectum* has been shown to be formed in soils. It is stable in the rhizosphere of crop plants and is known to impair membrane permeability at fairly low concentrations. Its preferential avidity for iron (Fe''') may also cause the removal of this metal from porphyrin moieties (Malini, 1963). These primary changes in osmoregulation of host cells following toxaemia is followed by an increased conductivity and accumulation of electrolytes in the cells resulting in an increased ionic imbalance in the infected tissues (Sadasivan and Kalyanasundaram, 1956). Increased tissue contents of calcium, magnesium and manganese and a very much depleted potassium content and increased oxygen uptake mark the major metabolic changes (Kalyanasundaram and Charudattan, 1966; Sadasivan and Kalyanasundaram, 1966). In contrast to the tissue-non-specific fusaric acid, recent work on the tissue specific toxin victorin isolated from *Helminthosporium victoriae*, responsible for an oat disease syndrome, showed that it affects only victoria variety of oats. The victorin-treated tissues of the susceptible and resistant oat leaves showed that transpiration of both plants was reduced. It also showed that the leachates of the susceptible tissues contained sizable quantities of nitrogen, amino-acids, carbohydrates and inorganic phosphorus compared to the untreated tissues. In addition, as in the case of fusaric acid and the cotton tissue, there was a four-fold loss in potassium (Luke and Freeman, 1965, 1967). Victorin incites various metabolic changes in susceptible variety of oats but has no effect on the resistant variety. Toxin treatment enhanced malic acid and citric acid synthesis but had no effect on aconitic and succinic acids. This enhanced synthesis of malic acid is, in fact, released from the mitochondria and acts as a buffering agent against excess cations that leak from the vacuole. Although victorin causes numerous metabolic disturbances (Manchey and Wheeler, 1965; Wheeler, 1968), its primary mechanism of action is not fully known (Luke and Freeman, 1967). Possibly, the plasma membrane could be the site of action of victorin. In first-leaf homogenates of healthy 9–12

day old seedlings of sucseptible oat variety to victoria blight, enzymic oxidation of ascorbate was found only in the cell wall fraction. When such leaves were treated with victorin solutions, enzymatic oxidation of ascorbate was unaffected in wall fractions but was detected in non-wall fractions after treatment. Susceptible leaves treated with victorin contained more RNA than controls but no change was found in the DNA content. An inhibitor of DNA-dependent RNA synthesis (actinomycin D) did not prevent victorin-induced changes in respiration or in the activity and localization of ascorbate oxidation.

(iii) *Onward march to systemic fungicides.*—Looking at exudation as a fundamental problem, the total volume of exudate from excised apical root segments was found to be very different between cotton, sunflower, kidney bean, castor bean and corn. It varied from 40 μl/cm^2 in 45 hr (of root surface exposed to experimental solutions) in cotton to 12 μl/cm^2 in corn, the others showing intermediate values.

Studies on the passage of fungicides through the complex chemical and osmotic barriers in higher plant tissues have shown that active transport across cells is influenced by the type of chemical, the solvent used, area of application, dosage and relative humidity. The new class of systemic fungicides β-amino-ethyl-aril-ketones when applied to bean and tobacco has shown that penetration through roots and stems and translocation to leaves of C^{14} labelled substances occurs quickly. Although the potential usefulness of this compound in the control of *Uromyces* bean rust was considered obvious, the systemic migration of this compound occurs not only into a restricted treated area of the infected leaf but also gets systemically generalized as shown by autoradiography (Pellegrini *et al.*, 1965). In leaves, passive diffusion through cuticle and epidermis into different areas of palisade and spongy mesophyll also occur independently of the vascular mass flow. This non-specific permeation mechanism which depends on solubility of substances in membrane components of cells possibly indicates the presence or absence of sensitive reaction sites through which an agent can cause phytotoxic effects by inhibiting normal plant growth. An example of this is afforded by the lipid-soluble antibiotic, chloramphenicol which is absorbed by plant roots and translocated to leaves. This substance inhibits protein synthesis in bacteria but not fermentation, respiration or nucleic acid synthesis. Phosphorylation and respiration are not affected by chloramphenicol but it is suspected that it blocks the incorporation of amino-acids into nucleo-proteins as shown in carrot slices. Thus, studies on phytotoxicity of chloramphenicol, known to be an inhibitor of protein synthesis, have given a lead to the

understanding of processes affecting salt uptake and the mechanisms of active transfer of ions across root membranes (Sadasivan, 1966 *loc. cit.*). Permeability changes of this type brought about by the application of chemicals on plants are bound to have repercussions on leaching of plant nutrients especially in the tropics where the transpiratory losses are of a higher magnitude as compared to the temperate plants.

We have examined uptake and translocation of sulphanilamide and griseofulvin by rice plants resistant (Co 13) and susceptible (ADT 10) to the brown spot disease by *Cochliobolus miyabeanus* and their phytotoxic effects as indicated by nitrogen metabolism (Srivastava, 1966). The susceptible ADT 10 showed a decrease in amides in infected leaves treated with sulphanilamide and an increase with griseofulvin treatment. As regards amino-acids there was an increase in sulphanilamide-treated infected leaves as compared with griseofulvin treated infected leaves which showed almost half the amount seen in the correspondingly treated healthy plants. In contrast, in the resistant Co 13, there was very little or no difference in the amides or in the amino-acid content in health or under disease. We have also worked out the toxicity index in these two rice varieties with a number of compounds such as 8-hydroxyquinoline, EDTA, indole-3-butyric acid, thiourea, *para*-nitrophenol, sulphanilamide and griseofulvin. Both the resistant Co 13 and susceptible ADT 10 showed phytotoxicity at the second higher concentration used in all treatments and in addition in thiourea and *p*-nitrophenol the resistant variety showed significant phytotoxicity in both low and high concentrations. The point to make here is that phytotoxicity to a chemical varies with the genotype we are dealing with and no satisfactory rule of thumb could be evolved. In fact, comparing three sulpha compounds, sulphanilamide, sulphaguanidine and sulphadiazine, their accumulation in the plant was correlatable with transpiration in broadbean and it was shown that for the same amount of water transpired, sulphanilamide accumulated much more than sulphadiazine and sulphaguanidine accumulated the least. Comparative toxicity of five sulphonamides on tomato plants treated through the roots showed that sulphadiazine-treated plants showed least toxicity and sulphanilamide the most toxicity, the other three showing intermediate toxicity.

(iv) *Fungicides and host metabolic events.*—Following fungicide treatments many investigations have shown changed water relations and alterations in metabolic processes. Sodium trichloracetic acid inhibited both respiration and photosynthesis in tomatoes. Substituted ureas and carbamates at concentrations lower than those affecting cell division suppressed photo-

lytic reaction in isolated chloroplasts. Fungicides such as cyclohexamide, dyrene and zineb affect the synthesis of chlorophyll, deoxyribonucleic acid and ribonucleic acid. Phytotoxicity due to cyclohexamide was more severe on well-grown rather than on poorly-grown onion plants, whereas dyrene in addition induced decreased levels of DNA in leaves of onions. While DNA synthesis was inhibited by these fungicide sprays, DNA synthesis was increased abnormally following sprays with cyclohexamide. Although there were no differences in water content, dry matter and ash content of treated and control leaves, fungicides clearly influenced DNA and RNA syntheses (Shishiyama *et al.*, 1965). The antiblast antibiotic Blasticidin S showed accumulation of RNA in yeast. In excessive concentrations this antibiotic was phytotoxic to rice, soyabean, apple, pear, peach, cabbage, tomato and other plants. Aflatoxins are now known to suppress cell division and synthesis of DNA.

(v) *Pesticides and the tropics.*—Quite obviously, indiscriminate use of chemicals in the tropics utilizing the know-how evolved in temperate crop growing regions would seem harmful. A *prima facie* case has been made here for utmost caution in applying results of pest control obtained elsewhere as the behaviour of chemical toxicants in temperate regions would appear unsuited to different tropical climates where rapid translocatory, photosynthetic, transpiratory and generally accelerated metabolic rates prevail. Even in human medicine, where tissue response to most drugs, chemotherapeutants and antibiotics is fairly uniform with all races, habitats and climate, individual physiological incompatibility and rejection, not to mention immunoserological disturbances and allergies exist. It is, therefore, not surprising if residual effects of chemicals in plants, in soils, and in irrigation waters aggravate problems of phytotoxicity in areas with diverse cropping, soils and microclimates. Research in physical sciences is very much the same throughout the world but the problems biologists face are entirely different especially in the field of applied biology. Furthermore, with newer high yielding crop plants being rapidly introduced in most terrains in the developing regions, the uptake and residual chemical concentration in tissues and their longevity in soils in their primary toxic form are problems that have to be carefully weighed before accepting any new proprietary product, however promising its performance elsewhere has been. In general, the future aim should be the production of safer, less persistent and more specific fungicides and the fundamental and applied aspects of this will have to be generated right here. If these cardinal principles are not underlined and implemented in a well-planned programme of agroeconomy, we may, before

long, arrive at a point of no return especially in this rapidly moving field of chemicals for plant protection. For how long can our country continue to import pesticides from abroad?

III. The Complex Nature of Rice Blast

(i) *Nutrition and edaphic factors.*—Some of the early observations of the blast disease of rice caused by the fungus *Pyricularia oryzae* showed that an increasing soluble nitrogen content in the rice plant tissue was associated with susceptibility and furthermore, aspartic acid content was found to be low at the season of maximum susceptibility. These observations have been confirmed by applying higher nitrogenous fertilizers in the field when blast incidence increased in susceptible varieties while it had no appreciable effect on resistant varieties. Apart from these findings temperature also seemed to influence resistance/susceptibility. In the growing conditions at Madras, both in the field and in the glasshouse, blast incidence was greater in the cooler months of December and January, when days are cloudy with high relative humidity and soil and air temperatures are low. Resistant varieties become susceptible when grown in the hills under cool night temperatures (15–16° C). These preliminary findings when verified under stringent temperature regimes indicated that a night temperature of 20° C under controlled illumination for 14 hr with a 10 hr dark period favoured infection, whereas infection was poor even on susceptible varieties grown at night temperatures above 26° C. Thus, environmental temperature and nitrogen metabolism seem to be two interacting factors affecting disease incidence. Physiologically, low night temperatures are known to increase soluble nitrogen in leaves, particularly the amides, and restrict protein breakdown and turnover so that glutamine accumulates. We have shown the importance of glutamine in aiding spore germination in *Pyricularia oryzae*. In other situations high nitrate content has been shown to result in low nitrate reduction in leaves of plants kept at high night temperatures. It has been suggested that differences in the ability to utilize nitrate may be related to the genotypic control of nitrate reductase activity. Based on some of these observations on other systems, it appeared that resistance or susceptibility to blast was a phenotypic expression of the genotype night temperature interaction. The theme of this lecture would be, therefore, on nyctotemperature and host susceptibility to blast.

(ii) *Night temperature and pathogenicity.*—Spores of *Pyricularia oryzae* germinate well over a wide range of temperature from 20–30° C in water. However, glutamine exudates obtained from susceptible rice plants grown at 20° C aided spore germination, although glutamine was not strictly neces-

sary. Spores forming appressoria on leaves of rice plants were fewer in the high night temperature grown ones than in those grown in low night temperatures. Also, while a large number of spores penetrated the epidermal cells in the leaves of low temperature grown rice, penetration was rare under high temperature regimes. Both resistant and susceptible phenotypes are not infected when these plants are grown in night temperatures above 26° C but succumb to infection when grown at 20° C. The resistant variety, on the other hand, produces at 20° C only pinhead size resistant lesions compared to the susceptible variety. When excised leaves of susceptible rice variety Co 13 were treated with an exogenous supply of L-glutamic acid, a greater number of spots with a larger area was produced. DL-phenylalanine applied on such excised leaves tended to restrict the lesion size and also intensified defense reaction. Although the dependence of the fungus to thiamine, or one of its moieties, pyrimidine, was shown *in vitro*, there was no evidence that either thiamine or pyrimidine was available in nature in the guttate of the rice plant (Sadasivan *et al.*, 1965).

(iii) *Host surface and pathogenesis.*—The fungus spreads freely in the tissues of the susceptible variety of rice when grown at 20° C nyctotemperature but not in resistant varieties grown at the same temperature. An incompatible genotype nyctotemperature interaction seems to endow the resistant variety with a firmer cuticle. This may result in a retarded nitrogen metabolism and as a consequence in the shunting off of the carbon skeletons to structural metabolism of cuticle and wax synthesis. On the contrary, a compatible genotype night temperature interaction in the susceptible variety may stimulate nitrogen metabolism and lessen the chances of an optimum structural metabolic function building up the outermost defence barrier such as the cuticle.

(iv) *Changes in host metabolism*: (*a*) *Changes in nitrogen metabolism.*—The resistant variety of rice shows practically no increase in the nitrogenous constituents, whereas the susceptible variety did show an increase. In the resistant variety there was actually a decrease in the alcohol-soluble nitrogen content, total nitrogen and ratio of alcohol soluble nitrogen/alcohol-insoluble nitrogen (Ramakrishnan, 1966 *a*). Similarly, a decrease in free amino-acids and protein amino-acids was seen and this was ascribed to the soluble metabolites being removed from the infected leaves and translocated to other parts. The net gain in protein in the susceptible variety early in infection indicated an increase in protein synthesis following infection. Subsequently, such increases were not recorded indicating a block in protein synthesis with

advancing pathogenesis. This is also borne out by the fact that much accumulation of glutamine took place in the susceptible plant early in infection showing a higher protein metabolism over the healthy control. Accumulation of glutamine has also been recorded in rust-infected wheat leaves and also uredospores are known to be rich in glutamine. There are also claims that both glutamine and asparagine showed increase in rust-infected wheat.

The recorded increase in phenylalanine in infected plants perhaps indicates a tendency to synthesize aromatic compounds and this has parallel with wheat stem rust. Enhanced protein synthesis has also been noticed in sweet potatoes infected by *Ceratostomella fimbriata* and these new proteins have been assigned a role in resistance.

(*b*) *Changes in carbohydrates and phenolics.*—There was marked increase in the total carbohydrate in the susceptible leaf particularly in the 2nd and 4th day after infection but there was little or no increase in the infected resistant variety. In the resistant, total sugars showed a decrease in the susceptible leaf compared to the uninfected on the 1st day after inoculation but on the 2nd and 4th days after inoculation, total sugars registered a high value corresponding with a total rise in carbohydrates. The diminution in the quantity of the reducing sugars corresponded to a greater accumulation of polyphenols both in Co 13 and Co 29 varieties. This tendency had been shown in *C. fimbriata* disease of sweet potato. Although the phenolic content rose in both susceptible and resistant varieties of rice following infection with the blast fungus, this increase was more rapid in Co 29. Furthermore, the ratio nitrogen/phenol was much lower in the resistant variety *vis-a-vis* susceptible one. Phenols, therefore, seemed to be produced as a defence response to infection in this disease (Ramakrishnan, 1966 *b*).

(*c*) *Night temperature and host-parasite relations in blast disease.*—With increase in nyctotemperatures from 20 to 25 and 30° C, the susceptible Co 13 showed more and more resistance and consequently frequency, size and nature of lesions became less and less manifest. However, resistant Co 29 remained resistant at all three temperatures. Percentage spores forming appressorium was less on plants grown under high night temperatures as compared to plants grown at low night temperatures and this was more significant in Co 13. This has confirmed the observations of the Japanese workers where epidermis of rice grown in soils of low temperature contained a greater number of appressoria and the number of cells penetrated were greater than plants grown at high soil temperature. While the fungus successfully penetrates and generalizes freely in Co 13 plants grown at low night

temperatures (20° C), it either failed to penetrate, or, if it did, was arrested in the epidermis or in the succeeding layer of cells in the high night temperature treatments (25° and 30° C) and also in the resistant Co 29 (Ramakrishnan, 1966 *c*).

(v) *Nyctotemperature and the blast syndrome.*—The effects of low temperatures on rice plants seem to favour a greater nitrate reduction and synthesis of glutamine and amino-acids which not only accumulate but also aid greater synthesis of proteins. This accelerated tempo in nitrogen metabolism would then have repercussions on carbohydrate breakdown and respiration since these supply the energy and carbon skeletons for protein synthesis through amino-acids. This augmented nitrogen metabolism, however, may not be the sole determining factor in susceptibility. Nevertheless, accumulation of nitrate in rice leaves grown at high night temperatures due to failure of nitrate reductase activity would bring in its trail shunting of the carbon compounds to structural materials like cutin and waxes. Indeed, the nitrate reductase system of the susceptible rice variety Co 13 when grown in 20° C night temperature is stimulated resulting in the incorporation of nitrogen in the amide. However, such a stimulation is not noticed in the resistant variety Co 29 where the glutamine content is low as compared to the susceptible Co 13. The determining host resistance factors seem to operate through the nitrate reductase system which, in turn, is dependent on genetic inheritance. This has its repercussion on amide and amino-acid syntheses which again is known to be sensitive to environment, particularly temperature (Sadasivan, 1968).

(vi) *The new concept on phenotype/genotype/biotype interaction.*—Until two years ago much of what has been covered above indicated quite clearly that the increased susceptibility of a susceptible genotype to blast disease was a double interaction: a phenotypic expression of a genotype where the exposure of a given genotype to a low night temperature of 20° C for a period of 10–15 days would increase susceptibility and proneness to blast. The biochemical changes and sequence of events in the host metabolism that this environmental regime would bring about on the given genotype led us to believe that this was a double interaction.

Recent work by our group broke some new ground. There are two biotypes of *Pyricularia*, one operating at 20° C nyctotemperature and another at 15° C. Spore types of *Pyricularia* showed distinct morphological parameters. For example, isolates from the grass *Panicum repens* and rice showed both small and large spores, whereas those from the grasses *Leersia hexandra* and *Brachiaria mutica* showed predominantly small spores. There appears,

therefore, no species concept and what we are dealing with in *Pyricularia* are biotypes. Our investigations show that the small spore type seems to operate at 15° C and the large spore type at 20° C. This rather circumscribed and yet critical environmental temperature for the biotype to act and bring about host responses to infection by optimal or a keyed-up nitrogen metabolism at the particular temperature of 15° or 20° C, as the case may be, would seem to bring into play three factors instead of two. This may now be defined as a phenotypic expression of a genotype in relation to the biotype of the pathogen. However, there are other complicating factors as indicated by some of the recent data collected here. Isolates from the three grasses showed on rice differentials exposed to 20° C nyctotemperature that they had generally a limited or restricted pathogenicity. On the other hand, biotypes capable of infecting rice at 20° C nyctotemperature arise when these very grass isolates are passed through three cereals: *Triticum vulgare* (wheat), *Hordeum vulgare* (barley) and *Secale cereale* (rye). So, obviously the grass isolates undergo some genetic change indicating the existence of the 20° C biotype in grasses in nature. In other words, the bulk of the spore population in the grasses seem to be essentially of the 15° C biotype and what, indeed, happens when they are passed through the three cereals appear to be a process of selection when the 20° C biotype comes to light (Thiagarajan, 1970).

Our efforts so far have been directed to an understanding of the *Pyricularia* isolates (whether they are the 15° C or 20° C biotype) from the grasses *Panicum repens*, *Leersia hexandra* and *Brachiaria mutica* and their role in the triple interaction described here. What we have taken for granted in this scheme is that we were dealing with single genomes among these grass populations and the small or large spore samples examined were, in fact, derived from stable grass genotypes. It may well be that these common grasses, which have a wide distribution throughout this sub-continent, could turn out to have as much variability in a given population as between the extreme cases of rice differentials where the subtle differences in the degrees of infection have been evaluated. We are now looking forward to concentrating on this aspect of the work but grass systematics and cyto-taxonomy may have to rise to the occasion and lend support to the clearing up of the complexities of this problem.

IV. Plant Viruses and the Mycoplasma Syndrome

(i) *Virology—an interdisciplinary approach.*—During the past three decades the emphasis on plant viruses has shifted from descriptive symto-

matology, epidemiology and syndrome to one of studying the nature of the causal agent and the metabolic changes they bring about in the diseased host. The morphology of viruses, their composition, their longevity *in vitro*, virus-vector relationships, chemical methods of control of viral nucleic acids, all go to make the subject of absorbing interest not only to the plant pathologist and biochemist, but also to the entomologist, agronomist and plant breeder. I shall endeavour to bring some of the more recent advances in plant virology and leave out the better known symptomatology, transmission, viral strains and general problems of epidemiology.

(ii) *The nature of viruses.*—Except the algal viruses (which contain DNA) all other plant viruses (of higher plants and mushrooms) contain RNA. The RNA viruses so far analysed have nucleic acid with a molecular weight of the order of 2 million. Electron microscope studies and X-ray crystallography have shown that virus particles are not uniform in structure but contain a fragile core-thread of a nucleic acid (the 'genetic' material of the virus) surrounded by a protective protein coat. Different plant viruses show widely differing size, contain different proportions of ribose type of nucleic acid and the manner in which the types of protein are arranged also differ widely. For instance, tobacco mosaic virus is a hollow tube of spiralling protein sub-units embedding the spiralling thread of nucleic acid. The spiral has 49 protein units in every three turns and every one turn of the spiral has 49 nucleotides. It is now considered that each of these protein units is a coiled polypeptide chain containing 158 amino-acids and the sequences have been worked out. It is also now known that the nucleic acid thread contains more than 5,000 nucleotides (Bawden, 1966). Viral strains seem to have the same characteristics except that the sequences of amino-acids along the polypeptide chains are different. Applying our knowledge of the genetic code to viruses, it has been proposed that fewer than one-tenth of the nucleotides seem to be needed to code for the structural protein and, therefore, the virus is left with many nucleotides to code for other activities. It appears that a virus to be pathogenic, its ability to code for structural proteins is itself not enough. The discovery of the satellite virus has brought to the surface this point. The satellite virus is the smallest known plant virus at present and possibly has very few nucleotides to spare after coding for its structural protein. This virus fails to cause symptoms and is unable to multiply in the host tissue unless aided by the large particulate tobacco necrosis virus with which it was once existing in nature as a 'contaminant'. Virus multiplication in plant cells primarily represents a derangement of the nucleic acid metabolism of the host cell with secondary effect on protein

metabolism. The satellite virus was referred to as unable to multiply *in vivo* unaided by tobacco necrosis virus. The name 'activator' has been given to tobacco necrosis virus and the association is now called 'activation'. The activator is often found alone and it multiplies indefinitely in plants without initiating the production of satellite virus which has to be introduced as such (Kassanis, 1966).

On the broader question of virus replication *in vivo* it is now suggested that the particle may be 'disrobing' and releasing its nucleic acid somewhere in the cell, the 'rerobing' site is perhaps the nucleus and the ribozomes are the likely site for protein synthesis. The complete virus particle would then be the assembling of the components synthesized *in vivo*.

Another landmark has been the discovery of a naked virus consisting only of RNA nucleic acid in the potato spindle tuber virus (Diener and Raymer, 1967). This and the rice dwarf virus (Miura *et al.*, 1966) have been shown to be double stranded RNA viruses (as against plant viruses so far described as having single standed RNA). The algal viruses which form a very distinct group of DNA viruses is another important field of research activity. The progress of lysis along the algal filament (*Plectonema boryanum*) showed that the period of time required to lyse cells is approximately 45 seconds, an incredibly short period. It has been suggested that the viral DNA is formed in the nucleoplasm. From there it is thought to move into spaces between the photosynthetic lamellae where large helices begin to develop. These in turn move into the virogenic stroma where the helix receives its protein coat and is 'compressed' into mature particles (Smith and Brown, 1967).

(iii) *Reconstituted viruses.*—The rod-shaped tobacco mosaic virus was first to be reconstituted from its protein and RNA components. Since then several spherical viruses have been reconstituted. Low molecular weight protein isolated from the small isometric plant viruses known as cowpea chlorotic mottle and brome mosaic virus, reaggregate in certain conditions to form spherical shells similar in size to the respective original virus particles and called " pseudo-top component". The surfaces of both viruses and their top component particles were very similar indicating that the protein sub-units alone have assembled themselves into a shell of virtually the same structure as that of the virus particles.

(iv) *Interferon.*—Before we pass on to a consideration of the recent discovery of the role of mycoplasma in plant etiology and syndrome we could

devote some time to the new thinking in animal viruses. The term 'Interferon' was introduced after some observations on cells of vertebrates which indicated the presence of a substance that interfered with the replication of virus. This substance seemed to assist the host to resist and to recover from virus infections. Interferon seemed to act as a 'regulator' both in infected and normal cells. In other words, it is an antiviral substance produced in vertebrate cells in response to virus infection (Isaacs, 1962). Viral interference has been noticed among plant, bacterial and animal viruses and is essentially a phenomenon in which cells infected with one virus acquire resistance to super-infection with many other viruses. Once interferon is formed within cells, it is rapidly liberated into the surrounding medium so that it can protect neighbouring cells. The question now engaging the attention of workers in this field is to determine the exact role interferon might play in the metabolism of the normal cell.

Interferon was first detected in cells treated with inactivated virus and virus multiplication, therefore, did not appear necessary in order to stimulate cells to produce interferon. Viral protein and viral nucleic acids were mainly suspected to be the seat of production of interferon but of the two, viral nucleic acid is, perhaps, to be incriminated more. At present, interferon is thought to play a role in the economy of the normal cell presumably connected with the synthesis of certain cellular nucleic acids. A variety of chemically and biologically heterogeneous substances have been found that would stimulate interferon formation in animals and offer protection against diseases. These substances ranged from killed viruses, bacterial endotoxins, micro organisms and rickettsiae to fungal metabolic products such as helemine and statolen and poly anions of large molecular weight. Preferential inactivation of viral RNA and non-virus protein seems to lead to inactivation of the capacity of the virus to induce interferon. Double-stranded RNAs from several viruses and yeast as well as synthetic double-stranded RNAs seem to induce interferon. The RNA copolymer poly (I.C.) is one of the most potent inducers of interferon in animals and has high activity in the prevention of experimental virus infection (Harris, 1970).

Plant pathologists have been experimenting with plant cultivars bred for resistance to fungal diseases. Certain low molecular weight substances collectively termed phytoalexins have been noticed to be formed in plants consequent on infection. Some of them have been characterised. They range from chromanocoumarans to furfurals and, in fact, many other phenolic compounds have also received attention in this race for understanding defence

mechanisms in plants. None of these efforts, however, have anything in common with the interferon concept in animal diseases.

(v) *Mycoplasma group of organisms.*—The first breakthrough in this area of mycoplasma (commonly called PPLO—pleuropneumonia-like organism after the first recognized species and now classified as *Mycoplasma* spp.) being the causal agent of plant diseases came from the Japanese pathologists (Doi *et al.*, 1967). They examined the phloem contents of plants infected with Mulberry Dwarf, Potato Witches' Broom and Aster Yellows (which were described as virus diseases) and reported that virus particles were not seen in mulberry affected with dwarf disease. Instead, in the sieve tubes and in the phloem parenchyma of diseased plants specific pleomorphic bodies were noticed which were spherical to irregularly ellipsoidal in shape and measured 80 to 800 mμ in diameter with two-layered limiting membrane (8 mμ) instead of a cell wall. Smaller bodies in the range of 100–250 mμ were nearly round and generally filled with ribosome-like granules. Gross morphology and fine structure of these bodies were somewhat similar to either the cells of *Mycoplasma* species or agents of Psittacosis-Lymphogranuloma–Trachoma group (PLT)-like micro-organisms. These mycoplasma-like bodies showed staining reactions for both DNA and RNA, whereas viruses of higher plants and mushrooms have so far been found to possess only RNA, although algal viruses, as already mentioned, are DNA viruses.

The mycoplasma hypothesis in so far as the Mulberry Dwarf disease is concerned seemed to be a favoured one as the specific bodies disappeared from the phloem of plants on treatment with tetracycline antibiotics. Presence of mycoplasma-like bodies in phloem has been confirmed also in preparations from Witches' Broom and *Petunia* infected with Aster Yellows (Davis *et al.*, 1968). Notwithstanding all these observations, the actual isolation and culturing of these organisms and then proving Koch's postulates would seem the next logical step and this has been recently achieved as will be evident below. Also, evidences are coming in of these bodies being detected in leafhoppers that act as vectors and, indeed, acquisition of the agent of Aster Yellows by leafhoppers was drastically reduced when infected plants were treated with chlortetracycline continuously for one week before exposure to the vectors.

The discovery of the Japanese scientists has been confirmed by a number of other workers (Hirumi and Maramorosch, 1969). Newer suspected mycoplasma involvement in what otherwise were confirmed viral infections are coming to light and at least 25 plant diseases have been reported till the middle

of 1970. The vexed Sandal Spike disease is one such. Mycoplasma-like bodies in Cactus virus X (in Witches' Broom diseased *Opuntia tuna*) have been demonstrated by electron microscopy (Casper *et al.*, 1970). Corn stunt, a yellows-type disease affecting corn (*Zea mays*) is transmitted by several species of leafhoppers. The causal agent of this disease considered to be virus earlier has now been shown to have mycoplasma-like bodies in diseased corn as well as in inoculative insect vectors. Purification and growth in artificial medium of a mycoplasma isolated from infected pea plant and also from the Rio Grand strain of corn stunt has been reported recently. The agent from corn apparently multiplied in cell-free medium. Leafhopper vectors (*Daubulus maidis*) that were infected with the pure cultures transmitted the agent to healthy corn plants and induced the corn stunt disease. It has also been demonstrated that the Rio Grand strain of corn stunt agent is pathogenic to the vector *D. eliminatus* and this perhaps establishes a new class of organisms that infect invertebrates (Chen and Granados, 1970). Electron microscopy of sections of intestines and salivary glands of the leafhopper *Macrosteles fascifrons* which had been exposed to clover phyllody-affected plants revealed the presence of a pleomorphic organism similar to *Mycoplasma* species. The salivary glands of exposed leafhopper, removed 42 days after the start of the acquisition access period, showed a large concentration of mycoplasma cells. Some acrini were found completely filled with the organism. The organism showed budding and it is suggested that this may be the multiplying process (Sinha and Paliwal, 1970).

V. *PETITIO PRINCIPI:* WHY PLANT PATHOLOGY ?

The most crucial part of a lecturing programme is when one settles down to summarizing the many aspects touched upon during the lectures. What really seems important is to stress the need for teaching the subject of plant pathology as an integrated science in Indian universities. It will be at once apparent that one has to be an initiated mycologist, a knowledgeable microbiologist and a keen physiologist before he hopes to be a respectable plant pathologist ! Then, why not plant pathology? At a time when the newer universities are plumping in for the currently glamour subject of molecular biology those in administration who peruse these 'notes' would realize that, for a developing nation with progressive schemes in an expanding and ambitious agricultural programme, a better understanding of plant pathogens would be as relevant as unravelling the mysteries of the genetic code or reconstituting a virus *in vitro*. Indeed, students of the conventional botany courses would, I hope, appreciate that plant pathology can also be

no less satisfying and stimulating intellectually as the most turse exposition on the origin and role of nucleic acids or the functioning of the mitochondria or the excitation of the chlorophyll molecule by electrons.

If I would have succeeded, even in a small measure, in enthusing the young students I had the privilege of lecturing to in three universities under this National programme of specialist lectures, to become 'pathologically minded' my efforts would have been worthwhile.

REFERENCES

Andal, R., Bhuvaneswari, K. and Subba Rao, N. S. "Root exudates of paddy," *Nature, Lond.*, 1956, **178**, 1063.

Bawden, F. C. .. "Some reflections on thirty years of research on plant viruses," *Ann. appl. Biol.*, 1966, **58**, 1–11.

Bhuvaneswari, K., Sadasivan, T. S. and Sulochana, C. B. "Rhizosphere microflora in relation to soil-borne diseases of plants," In *Advances in Agricultural Sciences, Golden Jubilee Commemoration Volume*, Madras Agricultural College, Coimbatore, 1965, pp. 538–48.

Casper, R., Lesemann, D. and Bartels, R. "Mycoplasma-like bodies and viruses in *Opuntia tuna* with Witches' Broom disease," *Pl. Dis. Reptr.*, 1970, **54**, 851–53.

Chen, T. A. and Granados, R. R. "Plant pathogenic mycoplasma-like organism: Maintenance *in vitro* and transmission to *Zea mays* L.," *Science*, 1970, **167**, 1633–36.

Chinnayya, E. J. and Agnihothrudu, V. "Rhizosphere microflora of plants growing in different ecological habitats," *J. Madras Univ.*, 1953, **23B**, 182–92.

Davis, R. E., Whitcomb, H. F. and Steere, R. L. "Remission of Aster Yellows disease by antibiotics," *Science*, 1968, **161**, 793–95.

Diener, T. O. and Raymer, W. B. "Potato spindle tuber virus: A plant virus with properties of a free nucleic acid," *Ibid.*, 1967, **158**, 378–81.

Doi, Y., Teranaka, M., Yora, K. and Asuyama, H. "Mycoplasma- or PLT group-like micro-organisms found in the phloem elements of plants infected with Mulberry Dwarf, Potato Witches' Broom, Aster Yellows, or Paulownia Witches' Broom," *Ann. Phytopath. Soc. Japan*, 1967, **33**, 259–66.

Hanchey, P. and Wheeler, H. .. "Ultrastructural changes in susceptible oat tissue treated with victorin," *Phytopathology*, 1965, **55**, 1060–61.

Harris, M. .. "Interferon: Chemical application of molecular biology," *Science*, 1970, **170**, 1068–70.

Hirumi, H. and Maramorosch, K. "Further evidence for a mycoplasma etiology of Aster Yellows," *Phytopathology*, 1969, **59**, 1031.

Isaacs, A. .. "The role of interferon," *Endeavour*, 1962, **22**, 96–98.

Kalyanasundaram, R. and Charudattan, R. "Toxins and plant diseases," *J. sci. industr. Res., India*, 1966, **25**, 63-73.

Kassanis, B. .. "Properties and behaviour of satellite virus," in *Viruse., of Plants*, eds. A. B. R. Beemster and J. Dijkstra, Norths Holland Publishing Co., Amsterdam, 1966, pp. 177–87.

Lakshmi-Kumari, M. .. "Rhizosphere microfloras of *Dolichos* infected with DEMV," *Proc. Indian Acad. Sci.*, 1964, **60 B**, 116–27.

Leelavathy, K. M. .. "Amino-acids and sugars in the exudates from germinating seeds of rice," *Ibid.*, 1970, **72 B**, 81–90.

Luke, H. H. and Freeman, T. E. "Effects of victorin on Krebs cycle intermediates of a susceptible oat variety," *Phytopathology*, 1965, **55**, 967-69.

———— .. "Relationship between the molecular structure of cytokinins and victorin activity," *Ibid.*, 1967, **57**, 820.

———— .. "Effects of cytokinins on the absorption of victorin," *Ibid.*, 1968, **58**, 258–59.

Malini, S. .. "Heavy metal chelates of Fusaric acid: *In vitro* spectrophotometry," *Phytopath. Z.*, 1963, **57**, 221–31.

Miura, K., Kimura, I. and Suzuki, N. "Double-stranded Ribonucleic acid from Rice Dwarf Virus," *Virology*, 1966, **28**, 571–78.

Natarajan, K. .. "Studies on the rhizosphere mycoflora of some crop plants," *Doctoral Thesis*, University of Madras, 1969.

Pellegrini, G., Bugiani, A. and Tenerini, I. "Systemic properties of a new class of fungicides," *Phytopath. Z.*, 1965, **52**, 37–48.

Rajagopalan, K. and Bhuvaneswari, K. "Effect of germination of seeds and host exudations during germination on foot-rot disease of rice," *Ibid.*, 1964, **50**, 221–26.

Ramachandra Reddy, T. K. .. "Foliar spray of urea and rhizosphere microflora of rice," *Ibid.*, 1959 *a*, **36**, 239.

———— .. "Rhizosphere microflora of pteridophytes," *Curr. Sci., India*, 1959 *b*, **28**, 113–14.

———— .. "Plant treatment in relation to rhizosphere effect. I. Pretreatment of roots of rice seedlings to certain chemicals and antibiotics on the rhizosphere microflora," *Pl. Soil*, 1968 *a*, **28**, 347–56.

———— .. "Plant treatment in relation to the rhizosphere effect. II. Foliar applications of certain chemicals and antibiotics in relation to the rhizosphere microflora of rice (*Oryza sativa* L.)," *Ibid.*, 1968 *b*, **29**, 102–13.

Ramakrishnan, L. .. "Studies in the host-parasite relations of Blast disease of rice. I. Changes in N-metabolism," *Phytopath. Z.*, 1966 *a*, **55**, 297–308.

———— .. "Studies in the host-parasite relations of Blast disease of rice. II. Changes in carbohydrates and phenolics," *Ibid.*, 1966 *b*, **56**, 279–87.

Ramakrishnan, L. .. "Studies in the host-parasite relations of Blast disease of rice. III. The effect of night temperature on the infection phase of Blast disease," *Phytopath, Z.*, 1966 *c*, **57**, 17–23.

Sadasivan, T. S. .. "Rhizosphere microfloras," in *Proceedings of the Summer School of Botany, Darjeeling*, eds. P. Maheshwari, B. M. Johri and I. K. Vasil, University of Delhi, 1962, pp. 363–69.

———— .. "Phytotoxicity," *Curr. Sci., India*, 1966, **35**, 273–76.

———— .. "Nitrogen metabolism and resistance to facultative parasites," In *Biochemical Regulation in Diseased Plants or Injury*, The Phytopathological Society of Japan, Tokyo, 1968, pp. 239–52.

——— and Kalyanasundaram, R. "Spectrochemical studies on the uptake of ions by plants. I. The Lundegårdh flame technique of ash analysis of toxin/antibiotic invaded cotton plants," *Proc. Indian Acad. Sci.*, 1956, **43 B**, 271–75.

———— "Phytotoxins and toxaemia," In *Beiträge zur Biochemie und Physiologie von Naturstoffen* (Prof. Kurt Mothes Commemoration Volume), Gustav Fischer Verlag, Jena, 1966, pp. 379–86.

——— and Saraswathi-Devi, L. "Vivotoxins and uptake of ions by plants," *Curr. Sci., India*, 1957, **26**, 74–75.

———, Suryanarayanan, S. and Ramakrishnan, L. "Influence of temperature on Rice Blast Disease," In *The Rice Blast Disease*, The Johns Hopkins Press, Baltimore, 1965, pp. 163–71.

Saraswathi-Devi, L. and Sadasivan, T. S. "Cotton wilt and calcium accumulation," *Experientia*, 1961, **17**, 554–55.

Shishiyama, J., Fukotomi, M. and Akai, S. "Effect of some fungicides on the synthesis of chlorophylls deoxyribonucleic acid and ribonucleic acid in onion leaves," *Phytopathology*, 1965, **55**, 844–47.

Sinha, R. C. and Paliwal, Y. C. "Localization of a *Mycoplasma*-like organism in tissues of a leafhopper vector carrying clover phyllody agent," *Virology*, 1970, **40**, 665–72.

Smith, K. M. and Brown, R. M. Jr. "Ultrastructural and time-lapse studies on the replication cycle of the blue-green algal virus LLP-1" *Ibid.*, 1967, **31**, 329–37.

Srivastava, S. N. S., .. "Uptake and translocation of sulphanilamide and griseofulvin by the rice plant," *Curr. Sci., India*, 1966, **35**, 327–29.

Sullia, S. B. .. "The fungicide Kitazin and the mycoflora of rice," *Proc. Indian Acad. Sci.*, 1969, **69 B**, 295–308.

Sulochana, C. B. .. "Amino-acids in root exudates of cotton," *Pl. Soil*, 1962 *a*, **16**, 312–326.

———— .. "Vitamins in root exudates of cotton," *Ibid.*, 1962 *b*, **16**, 327–34.

Sulochana, C. B. .. "Cotton roots and vitamin-requiring and amino-acid requiring bacteria," *Pl. Soil*, 1962 *c*, **16**, 335–46.

——————— .. "Mycoflora of the root region," *Curr. Sci., India*, 1968, **37**, 188–90.

Thiagarajan, S. .. "Studies on *Pyricularia* with reference to host-parasite relationships," *Doctoral Thesis*, University of Madras, 1970.

Wheeler, H. .. "Effect of victorin on transpiration of resistant oat leaves," *Phytopathology*, 1968, **58**, 1071–72.