STUDIES ON THE NODAL INFECTION OF RED ROT OF SUGARCANE

BY

K.I. WILSON

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CERTIFICATE

This is to certify that the thesis entitled "Studies on the nodal infection of red rot of sugarcane" submitted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Mycology and Plant Pathology, Faculty of Post-Graduate School, Indian Agricultural Research Institute, New Delhi is a faithfull record of the bonafide research work carried out by Shri K.I. Wilson, under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

25/x/63
(B.L. Chona)
HEAD OF THE DIVISION

Approved by:

Chairman: .....
(B.L. Chona)

Members: 1. .....
(D.N. Srivastava)

2. .....
(N.B. Das)

3. .....
(M.L. Magoon)

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I. INTRODUCTION

Of about 35 diseases that are known to affect the sugarcane crop in India, Red Rot caused by Colletotrichum falcatum Went (Glomerella tucumanensis (Speg.) Arx and Muller) is by far the most important. The disease has often assumed serious proportions and become a limiting factor in the successful cultivation of several high-yielding cane varieties, particularly in Northern India, which is the main cane tract of the country.

Detailed investigations regarding the sources and modes of red rot infection, conducted by Chona (1950) have clearly revealed that even a healthy sugarcane crop grown in healthy field, from healthy setts can become infected with red rot later in the season, through the nodal regions of the cane. Infection through the nodal region mainly takes place through irrigation or rain water containing the spores of the fungus, or by the washing down of the spores produced on the leaf midrib lesions, with rain or excessive dew, into the cavity between the stalk and leaf-sheath of the cane. Nodal infection is the chief mode of field infection of the sugarcane crop in nature and is responsible for the annual recurrence of the disease and consequent crop failures, since the nodal infected setts are likely to pass undetected at the time of selection of setts for planting (Chona, 1951).

It has been shown that the power of nodal infection is not possessed by all the isolates of the red rot fungus and

hence the predominance of only such isolates that possess the power of infecting through the nodal region could cause any appreciable secondary infection of the cane crop and bring about an epidemic. Furthermore, great differences have been observed among different sugarcane varieties regarding their susceptibility to this mode of infection (Chona, 1956a); but little is known at present as to why a particular variety is susceptible or resistant.

The present investigation was undertaken with a view to study the extent of red rot infection through the nodal region and to probe into the nature of resistance to nodal infection and spread of the pathogen in the cane tissues and also to find out, if possible, some fairly rapid laboratory methods for testing the resistance of sugarcane varieties to red rot.

The results obtained are presented in this thesis.

II. REVIEW OF LITERATURE

Red rot of sugarcane was first reported from Java by Went (1893) who named the causal organism as Colletotrichum falcatum Went. He proposed the name "Rood Snot" (red smut) for the disease. In India, Barber (1901) first reported a severe outbreak of the disease in the Godaveri delta of Madras State. Butler (1906) proposed the name "Red Rot", which is now the universally accepted name of the disease.

Though the ascigerous stage, Physalospora tucumanensis (Glomerella tucumanensis) was first collected and described from Argentina by Spegazzini as early as 1896, Carvajal and Edgerton (1944) were the first to establish its connection with the imperfect stage, Colletotrichum falcatum Went.

Chona and Srivastava (1952) produced the perfect stage of the fungus for the first time in India, by inoculating the conidial stage of the fungus on dry autoclaved leaf blades and leaf-sheaths of sugarcane. Later, in 1953, Chona and Bajaj reported the occurrence of the perfect stage under naturel conditions.

Arx and Muller (1954) transferred the ascigerous stage of Colletotrichum falcatum Went to the genus Glomerella and renomed the fungus as Glomerella tucumanensis (Speg.) Arx and Muller.

Avenues and modes of red rot infection at the nodal region:

Went (1893) stated that the place of insertion of the

leaf-sheath at the node was permeable to natural infection by Colletotrichum falcatum. Howard (1903) made inoculations at the leaf-bases and obtained infection in some cases.

Butler and Hafiz Khan (1913) obtained infection through the adventitious root-eyes and injured buds, by artificial inoculation. Inoculations made on uninjured bud scales caused slight reddening of the scale at the margins after one month, with numerous hyphae in the reddened part. The underlying bud-layers were only faintly discoloured and very few hyphae had entered them, while the deeper layers remained free from infection. As the progress of infection was very slow, they doubted whether the young shoot would be reached before the outer layers had withered away or lost contact with it. They observed that the adventitious root-eyes were more easily infected by the fungus. According to them, the leaf-scars are not readily penetrated by the fungus, and since the leaf-scars are not normally exposed until the leaf has completely withered, they were not considered as important point of entrance.

Freise (1930) stated that C. falcatum penetrated through the rudimentary roots. Abbott (1938) reported that infection of the growing canes occurred in some varieties through the root primordia, but was limited largely to certain very susceptible varieties, like P.O.J. 213 and P.O.J. 2714. Chona (1939) observed secondary infection through the nodal regions of canes, in the red rot epidemic areas of North Bihar, chiefly on the variety Co. 213. Chona and Padwick (1942),

after careful examination of thousands of canes in the epidemic areas of Eastern Uttar Pradesh and Northern Bihar reported that, considerable amount of secondary infection occurred at the upper or middle region of the cane, probably through the nodal region, in the entire absence of any basal infection or borer injury.

Wiehe (1944) opined that red rot infection might occur at the growth-ring of bent canes; infection coinciding with the outer bend of the growth-ring.

Chilton *et al.* (1947) observed infection of the root-bands and scales covering the buds. Steib (1949) reported that the buds with infected scales developed red rot under favourable conditions.

Ghose (1950) conducted detailed investigation on the sources and modes of red rot infection and after careful examination of canes artificially infected at the nodal region reported that infection took place chiefly through the leaf-scar, root primordia and growth-ring; but very seldom through the eye-buds. Steib and Chilton (1951) obtained the fungus on isolation from the leaf-scar, bud and root-band tissues of the nodes of apparently healthy stalks of sugarcane varieties susceptible to red rot disease. Field observations and experiments conducted by them indicated that the fungus first developed behind the leaf-sheaths as they pulled away from the stalk and then spread and finally infected the nodal tissues.

Sanchez-Navarrete (1962) recovered the fungus from the leaf-scars, leaf-sheaths, internal tissues, bud scales and parts of undergournd shoots of 7 sugarcane varieties.

Relation of host exudates to infection:

Brown (1922) observed leaching of nutrients by passive exosmosis, from the surface of certain plant structures which in most cases stimulated the spore germination of Botrytis cinerea. The exudates from certain plants, however, reduced spore germination. Sattar (1933) reported that the susceptibility of gram plants to blight disease caused by Ascochyta rabiei, increased with age, being greatest at the flowering and fruiting stages, during which time the plants excreted the largest amount of malic acid from the glandular hairs, which favoured the spore germination of the fungus. Kovacs and Szecke (1956) studied the effect of leaf excretions from wheat, red clover, chilli, tomato and suger-beet on the spore germination of Botrytis cinerea, Ascochyta pisi and Puccinia triticina. They concluded that the excretions exerted either a stimulatory or inhibitory influence on spore germination, varying with the concentration and the susceptibility of the host plant. Spencer et al. (1957) detected water-soluble phenolic compounds, possessing fungistatic properties, in the exudates of Vicia faba.

Buxton (1957) noticed that the quality of exudate from the roots of different pea varieties could be correlated

with their resistance or susceptibility to the pea wilt fungus, Fusarium oxysporum f. pisi.

Weintraub et al. (1958) demonstrated a chemical stimulation of germination of the spores of Pirlcularia oryzae in the dew and guttation liquid collected from rice leaves. Sureyanarayanan (1958) identified glutamine in the guttates of rice leaves, which stimulated the spore germination of P. oryzae.

Schroth and Snyder (1961) identified glutamic acid, aspartic acid, asparagine, glucose, sucrose, maltose and fructose in the root exudates of bean, all of which stimulated the chlamydospore germination of the bean root rot fungus, Fusarium solani f. phaseoli.

Orellana and Thomas (1962) analysed the water leachates of intact castor bean capsules, in order to study their relation to the varietal susceptibility to capsule mold, Botrytis cinerea. They observed that high, moderate, and low susceptibility of immature capsules was associated with a low amount of leachable sugar. They concluded that the differences in susceptibility could not be explained by qualitative differences in the constituents of the pericarp.

Mayeaux and Colmer (1960) demonstrated the presence of varying amounts of carbohydrates in the "leaf-sheath water" (water collected in the cavity between the leaf-sheath and stalk) of sugarcane. They reported that as the carbohydrate content increased, there was a corresponding increase in the

total number of micro-organisms in the leaf-sheath water. They were, however, not able to detect the presence of any amino acid in it. Pritam Singh (1962, unpublished) demonstrated the exudation of nutrients by passive exosmosis, from the nodal region, leaf-sheath and leaf-midrib of sugarcane, which stimulated the spore germination of C. falcatum.

Relation of nutrients in the host with disease incidence:

Availability of certain particular nutrients in the host and the ability of the pathogen to derive those nutrients from the host tissues have been considered to be important factors in determining the parasitism of pathogenic micro-organisms.

Leach (1923) working with Colletotrichum lindemuthianum concluded that the pathogen required specific nutrients which were furnished only by the susceptible host. He suggested the possibility of metabolites being present in different steriosomeric forms and that the ability of the parasite to use only certain forms might be the basis of resistance.

Vasudeva (1930) working on the factors responsible for the failure of Botrytis allii to attack apple, made a significant observation that the fungus could be made to parasitise apple by adding to the inoculum a small dose of certain soluble nitrogenous substances like asparagine, ammonium salts, etc. Apparently, resistance of apple to this fungus could be attributed to its low nitrogen content.

Chona (1932) observed that Fusarium coeruleum normally unable to parasitise apple could be made to do so, to a certain extent, when the inoculum was supplied with a trace of soluble nitrogen.

Correlation between aggressiveness and nutritional requirements has been demonstrated for various plant pathogens, with the aid of biochemical mutants. Garber (1954) and Garber and Shaeffer (1957), using mutants of Erwinia aroideae deficient for specific amino acids, to study the correlation between virulence and the presence of required nutrient in the host tissue, observed that the mutants attacked slices of certain vegetables only when adequate amounts of the required nutrients were present at the infection-site.

Kline et al. (1957) working with Venturia inaequalis concluded that the non-pathogenicity of certain mutants of the fungus deficient for certain specific substances was due to their inability to obtain them from the resistant host. Addition of the required nutrient to the surface of inoculated apple leaves restored the pathogenicity wholly or partially in some of the biochemical mutants.

Bajaj (1962, unpublished) showed that the virulence of an artificially induced mutant of C. falcatum, deficient in para-aminobenzoic acid, could be partly restored by incorporating the above mentioned vitamin into the sugarcane plant, through the roots.

Van Gundy and Walker (1957) while working on angular leaf-spot of cucumber caused by Pseudomonas lachrymans observed a consistent correlation between the amino acid nitrogen content of leaves and the severity of the disease and believed that the amino acid content of the leaf tissue was a primary factor in determining the susceptibility of cucumber leaves to infection.

Gallegly and Niederhauser (1958) working with the late blight fungus, Phytophthora infestans on potatoes, found that high levels of nutrition increased the amino nitrogen content of the leaves, thus making the tissue highly favourable for the growth of the pathogen.

Manocha and Chona (1963, unpublished) showed that with the increase in dose of ammonium sulphate fertilizer supplied to the sugarcane crop there was a corresponding increase in the nitrogen content of the juice, resulting in greater infection by the red rot pathogen.

Hedwiger and Hall (1961) studied the relation of pigmentation and free amino acid content with the resistance of watermelon to Colletotrichum lagenarium. They observed that the lesions resulting from infection were concentrated primarily on the dark-green stripes and that the upper cell layers of the dark-green stripes contained much higher quantities of citrulline and glutamine than the corresponding tissues of the light-green stripes.

Thomas and Orellana (1962), working on the resistance of sesame varieties and the pathogenicity of Pseudomonas Sesami, concluded that varietal reaction depended on differences in the concentration of certain amino acids and in the ratio of reducing sugars to those amino acids.

Nature of disease resistance:

Brown (1934) divided resistance into (1) Mechanical resistance and (2) Chemical resistance, the latter being divided into four groups namely, (a) acidity (b) presence of certain substances like gums, esters, tannins, oils, etc. (c) enzymes and (d) secretion of some active principle.

Edgerton (1956) recognised two types of resistance to red rot in sugarcane (i) morphological and (ii) physiological.

By "morphological" is meant the presence of structure or modifications of the plant which mechanically prevent the entrance of the pathogen or hinder itsspread through the various tissues. By "physiological" is meant the counteracting reactions of the protoplasm itself to the mycelium and spores of the pathogen.

According to Edgerton (1955) at least two types of structure modifications are involved in the morphological resistance: the modifications of the protective elements and the presence or absence of obstructions in the ducts of the vascular bundles. Varieties differed in regard to such

characteristics as thickness of the epidermis and cuticle, thickness of rind, thickness of the bundle-sheath, thickness of the scales protecting the buds on the stalks and the relative abundance of fibrovascular bundles in the stalk.

Atkinson and Edgerton (1937) reported that the spores of G. falcatum could travel through the fibrovascular bundles of sugarcane and such passage probably occurred when cut-stalks become infected at planting. Obstructions such as cross-walls in the ducts of fibrovascular bundles hindered or prevented the migration of conidia from internode to internode and might retard the longitudinal spread of infection.

Varma and Mitkal (1949) studied the continuity of conducting vessels by placing the cut-ends of fresh canes with leaves attached, in dilute "India-ink" solution and allowing the ink to ascend through the vascular system by transpiration pull. They observed that in the resistant groups (Co. 393 and Saccharum spontaneum) the percentage of continuous vessels was lower than that in the susceptible groups (Co. 213 and Co. 312). They did not find a single vessel continuous in the case of Saccharum spontaneum, which is known to be highly resistant to red rot disease. Tiwari (1957, unpublished) studied the correlation between the number of continuous conducting vessels in sugarcane varieties and the degree of resistance to red rot by drawing India-ink solution through cut-canes by means of suction pump

and corroborated the above findings.

Several workers have reported that the silica content of the plant could be correlated with disease-resistance. Miyaki and Aachi (1922) reported that the silica content of the rice plant could be correlated with the resistance to blast disease caused by Piricularia oryzae. Suzuki (1935) stated that resistant varieties of rice had more number of silicated epidermal cells. Studies conducted by Adyanthaya and Rangaswami (1952) indicated that a correlation existed between the distribution of silica in the epidermal tissues of rice leaves and the degree of resistance to blast disease. Chattopadhyay and Chakraborthy (1957) confirmed the findings of the above workers in the case of Helminthosporiose of paddy.

Ponnaiya (1951), while studying the silica content of Sorghum, observed that silica was deposited in most plant parts and that the maximum quantity of silica occurred in the leaf-sheath.

Ayres (1933) studied the silica content of sugarcane leaf-sheaths at different ages of the plant and showed that the silica content of the leaf-sheaths was maximum in 3-4 months old plants.

Physiological resistance to red rot according to Edgerton (1955) is perhaps the most important type of resistance and is not at present well understood.

Edgerton and Carvajal (1944) studied the host-parasite relationship in red rot of sugarcane. They concluded that the reaction of the protoplasm to the advancing mycelium involved many complex biological and chemical problems which concerned the question of resistance and susceptibility. In advance of the invading mycelium, the protoplasm changed in colour and a gummy dark-red material oozed out of the cells and filled the intercellular spaces. The zone in advance of the mycelium in which the changes occurred, became red because of the presence of a soluble dye which was absorbed by the cell-walls. The growth of the advancing mycelium was stopped, or atleast checked temporarily, at the red zone. The natural resistance of a variety depended on the rapidity of development of the red zone around the infected area. The red zone is formed quickly in resistant varieties and more slowly in susceptible varieties.

Chemical nature of disease resistance:

A good deal of literature is available on the chemical nature of disease resistance in plants. Cook and Taubenhaus (1911) found cases in which disease resistance was apprently correlated with the rate of tannin formation in the host cells. Cook and Wilson (1916) working with members of the genus Endothia in culture media found that in most cases growth was retarded by 0.8 per cent of tannin. They claimed

that, in general, tannin inhibited fungal growth and that the tannin content of the cells was one factor in the resistance of certain plants to the attack by fungal parasites.

Phenolic compounds, either present in the healthy tissues or produced as a result of infection by a parasite, have been reported to be important in determining disease resistance in certain plants.

Newton and Anderson (1929) observed some relation between the content of phenolic substances in wheat varieties and rust resistance. They suggested that rust resistance in wheat might be due to the liberation of phenols in the host cells upon entrance of the fungus. Link et al. (1929) and Link and Walker (1933) showed that the outer scales of coloured onions which are resistant to smudge disease contained water-soluble phenols like protocatechuic acid and catechol which were toxic to the spores of the smudge fungus, Colletotrichum circinans. Kargopolova (1936) reported that the cell-sap of wheat varieties highly resistant to leaf rust was characterized by a high concentration of phenolics of the protocatechuic acid type, while the sap of susceptible varieties was poor or entirely devoid of those substances.

Abbott (1936) conducted preliminary experiments to study the phenolic compounds in the internodes of sugarcane

varieties resistant and susceptible to red rot disease and observed that resistant varieties contained significantly higher quantities of phenolic compounds than the susceptible ones. Physiological investigations conducted by Evans (1941) indicated that the reaction of sugarcane to the invasion of Colletotrichum falcatum was associated with the amount in the former of an amino-phenol of the tyrosine type.

Work done by Dufrenoy (1942) showed that compounds like pyridoxin (Vitamin B_6) contributed to the resistance of sugarcane to red rot disease. At the margins of red rot lesions in the nodal region, greater amount of pyridoxin was present than in the healthy nodal portions.

Parthasarathy and Vijayasarathy (1958) while studying the phenolic content of sugarcane and spontaneum juices observed higher phenolic content in the juices of Saccharum spontaneum and Co. 285 than that in Co. 421, Co. 467, Co. 419, etc. They suggested that the higher phenolic content in spontaneums and Co. 285 might be of special physiological significance in the hardy and disease resistant character of these varieties.

Johnson and Schaal (1952) identified chlorogenic acid in potato tuber peels. They observed higher concentration of this phenol in potato varieties resistant to scab disease, caused by Stentomyces scabies, than in susceptible

ones and suggested a correlation between the chlorogenic acid content of potato peel and resistance to scab disease. An highly resistant variety contained 77 milligrams of chlorogenic acid per 100 grams of peelings, while a susceptible variety contained only 40 mg./100 gm. of the same tissue. Schaal et al. (1953) applied the ferric chloride test for detecting ortho-dihydroxy phenols in potato tubers and noted that resistant varieties showed the presence of higher amount of chlorogenic acid than the susceptible ones, as indicated by the intensity of green colour which varied with the degree of resistance. By applying 2 per cent aqueous ferric chloride over the freshly cut surface of a tuber and noting the production of green colour, they observed that in resistant varieties the colour reaction was greatest near the surface and that in some highly resistant varieties the chlorogenic acid was present throughout the tuber but with the greatest concentration occurring in the cells directly under the corky covering. Also they noted a tendency for chlorogenic acid to become concentrated around scab pustules, lenticels and injuries on a susceptible variety.

McLean *et al.* (1956) observed highly significant correlation between the cortical reaction of potato varieties to ferric chloride test and the resistance to wilt disease caused by *Verticillium albo-atrum*. Lee and Le Tourneau (1958) reported that the roots of potato varieties resistant to Verticillium wilt contained more chlorogenic acid than

susceptible varieties. They observed that the leaves contained more chlorogenic acid than the roots and stem. In vitro experiments conducted by them showed that chlorogenic acid was metabolized by Verticillium albo-atrum in liquid media. Penicylinder assays on solid medium showed that chlorogenic acid was more inhibitory to the organism in neutral or alkaline media than in acid media. Even at high p^H, large quantities (500 - 1000 ppm.) of chlorogenic acid were necessary for inhibition of the fungus.

McLean et al. (1961) studied the phenolic concentration in potato by using ferric chloride as histochemical test which indicated that high concentrations of Orthodihydroxy phenols were present in the vascular system. Concentrations of 7500 to 10,000 ppm., mostly in the form of chlorogenic acid, were present in the root stele, stem node and vascular system of young wilt-resistant potato plants. As the plants developed and matured the phenol concentration decreased more rapidly in susceptible varieties than in resistant ones.

Kinkel (1963) demonstrated the association of chlorogenic acid with physiological necrosis in potato tubers. He developed a histochemical test to detect the microscopic necrotic areas in the tuber tissues, by using nitrous acid and potassium hydroxide reagents.

Kuc et al (1955) observed that potato slices responded to inoculation with Helminthosporium carbonum, Ceratostomella ulmi and Fusarium oxysporum f. lycopersici, by producing a

substance inhibitory to the growth of those fungi. Later, Kuc et al. (1956) demonstrated chlorogenic and caffeic acids as fungistatic agents produced by potatoes in response to inoculation with H. carbonum.

Kirkham (1954) found that infusion of water-soluble phenolic substances into varieties of apple normally susceptible to scab disease caused by Venturia inaequalis, made the variety resistant to that disease. Studies conducted by him (1957) on the significance of phenolic metabolites in the nutrition of Venturia inaequalis and Venturia pirina showed that qualitative and quantitative variations in the polyphenolic metabolites, including differences in their relative proportions in relation to nitrogenous and other nutritional factors were of potential significance in the determination of pathogenicity and disease resistance. Flood and Kirkham (1959) observed that the reaction of Venturia species to phenolic compounds in culture medium could be modified by altering the composition of the basal medium in respect to its nitrogenous constituents. The inhibitory effect of phenolic compounds could be reduced by increasing the concentration of nitrogen. They concluded that the fungal reaction depended on a delicate balance between the concentration of phenolics and the amino acid nitrogen.

Hulme and Edney (1959) studied the phenolic substances in the peel of Cox's orange pipin apples with reference to storage rot caused by Gloeosporium perennans and noted that

a parallelism existed between increased susceptibility of the fruits and decrease in chlorogenic acid content of the fruit peel.

Kiraly and Farkas (1962) noted that infection of wheat plants with stem rust was associated with a marked increase in the phenolic content of the diseased leaves and in some resistant varieties the phenol accumulation was detected even before any visible symptom appeared. They reported that the resistant reactions to stem rust in wheat plants were associated with a consistently faster accumulation of phenolics and that the speed of phenol accumulation could be correlated with the infection type.

Echandi and Ferandez (1962) observed that coffee plants resistant to canker incited by Ceratocystis fimbriata contained much higher quantities of chlorogenic acid than susceptible varieties and that the concentration of chlorogenic acid in resistant varieties was well above the amount required to suppress the spore germination of the pathogen. They reported that resistant varieties of coffee plants contained upto 9000 ppm. of chlorogenic acid in their bark tissues and that the young branches had higher chlorogenic acid content than the older ones of the same species. They found that the germination of the endoconidia of the fungus was inhibited at 625 ppm of chlorogenic acid.

Quinones produced by the oxidation of phenols have been shown to contribute towards the disease resistance in

certain plants. The enzymes responsible for the oxidation of phenols are polyphenol oxidases of which two have been separated: (1) tyrosinase, which oxidises both mono - and polyphenols and (2) laccase which oxidises polyphenols only (Cochrane, 1958). The browning and blackening of potatoes, apples and many other plant tissues have been shown to be the result of oxidation of monohydric or polyhydric phenols.

Johnson and Schaal (1952) reported that upon tissue injury the enzyme tyrosinase present in potato tubers immediately oxidised chlorogenic acid to the quinone, which might be toxic to the pathogenic organism. The rate of quinone formation varied with the chlorogenic acid and tyrosine content of the tissues. Quinone formation in the area where chlorogenic acid was concentrated was also found to be greater in potato varieties resistant to scab disease. Schaal and Johnson (1955) observed that upon autoxidation, phenolic compounds like chlorogenic acid, caffeic acid, catechol and tetrahydroxybenzoin were effective in the inhibition of the growth of Streptomyces scabies. The inhibition increased with the increase in pH, which produced an increase in the rate of autoxidation.

Uritani and Akazawa (1955) indicated that the oxidative product of chlorogenic acid might contribute to the resistance of sweet potatoes to the infection by Ceratocystis fimbriata.

Le Tourneau *et al.* (1957) noted that, in culture, quinones were more inhibitory to the growth of *Verticillium albo-atrum* than phenols. Lee and Le Tourneau (1958) reported that the pH of the medium containing chlorogenic acid became alkaline as the fungus grew and quinone was formed, which was detected by the starch-potassium iodide test.

Kuc and Maxam (1959) reported that the inhibition of *Helminthosporium carbonum* by chlorogenic and caffeic acids was dependent on the medium and its pH. They suggested that the inhibition might be due to the formation of toxic phenols and quinone amino acid addition products and to the production of phenol oxidases.

III. MATERIALS AND METHODS

Isolate:

Isolate No. 423 of *Colletotrichum falcatum* Went, obtained from the stock culture of the Scheme for Research on Sugarcane Diseases, Indian Agricultural Research Institute, New Delhi was used in the present investigation. The culture was isolated in January, 1960 from diseased canes of variety Co. 356 obtained from Lakhimpur Kheri, Uttar Pradesh. The general cultural characters of the isolate are, light coloured, profusely sporulating with the production of abundant pink spore-masses on oat-meal-agar medium. The isolate has been proved to be highly virulent in the nodal infection tests conducted in the Sugarcane Disease Scheme, I.A.R.I.

Cane varieties:

The following six varieties of sugarcane grown in the Mycology Division area, Indian Agricultural Research Institute, were used for most of the studies: Co. 445, Co. 331, Co. 312, Co. 1181, Co. 1070 and Co. 285. Of these, Co. 1070 and Co. 285 have been proved to be moderately resistant to red rot infection (Chona, 1954, 1956). For certain experiments, only 4 varieties viz., Co. 445, Co. 331, Co. 1070 and Co. 285 were used depending on the availability of the cane material. As and when more canes were needed for experimentation, those were brought from the Agronomy Division Farm of the Institute.

Medium:

Oat-meal-agar medium having the following composition was used for the maintenance of cultures throughout the study.

Quaker oat	40 gm.
Agar agar	20 gm.
Distilled water, to make up.....	1000 ml.

Inoculum:

Ten to fifteen days old cultures were used for inoculation and other studies. The fungus was frequently subcultured at regular intervals, so that young active cultures were available for experimentation. Single-spore isolations were repeated at 5-6 months' interval for maintaining the purity of the isolate. The fungus was passed through the natural host, once in 10-12 months and reisolated, in order to ensure its virulence.

Spore suspension for inoculating the canes was prepared in sterilized water, from 2 weeks old cultures of the fungus. Spores from 1-2 Petri-dishes (depending on the intensity of sporulation) were used for every 100 ml. of sterile water.

Method of inoculation:

Standing canes were inoculated by pouring 1-2 ml. of the spore suspension into the cavity between the leaf-sheath and stalk, by means of a sterile pipette. Only the node supporting the 8th leaf-sheath from top was inoculated in each cane, since this leaf-sheath was generally green at

the time of inoculation. It was necessary to gently pull the leaf-sheath away from the stalk in order to facilitate the entry of the spore suspension and its reaching the nodal region.

Spore germination studies:

Spores from 10-15 days old cultures were suspended in sterile distilled water and filtered through muslin cloth in order to remove the larger mycelial fragments. The suspension was then centrifuged at 2000 rpm. for 20 minutes and the supernatant was poured off. Standard spore suspensions were prepared in approximately 5 ml. of each of the respective substrates or test solutions, having a concentration of 15-20 spores per microscopic field under the low power of the microscope. By means of a sterile pipette, 2 drops of the spore suspension were placed on clean, dry glass slides which were placed in Petri-dishes lined with moist filter paper and incubated at 25-27 ^{0}C for 16 hours. Four replicates were taken for each treatment and germination counts were taken by examination of 5 microscopic fields from each replicate and the average calculated. The criterion used for recording germination was the emergence of the germ tube to a length exceeding the width of the spore. Other characters like the type of germ tube and appressoria formation were also recorded.

Collection of leaf-sheath water:

Leaf-sheath water (the water accumulated in the cavity

between the leaf-sheath and stalk of sugarcane) was collected from 20 canes, growing in the field, at random, by means of a thin pipette and stored in the freezing chamber of a refrigerator.

Chromatographic detection of sugars, amino acids and phenols:

Samples of the materials to be analysed were spotted 4 cm. apart on the starting line drawn 3 cm. from one side of Whatman No. 1 filter paper sheet. The paper was then placed in the developing solvent, butanol-acetic acid - water (4:1:1), in the chromatographic chamber. After allowing the solvent to ascend for 18 hours the paper was taken out, air dried for 6 hours and then sprayed with the respective reagents mentioned below:

For the detection of amino acids, the chromatogram was sprayed with 0.1 per cent ninhydrin in n -butanol and heated at 90°C for 30 minutes in an electric oven (Block et al., 1959). For the detection of sugars, the chromatogram was sprayed with phloroglucinol solution prepared by mixing 25 ml. of glacial acetic acid, 1 ml. of concentrated hydrochloric acid and 2.5 ml. of 5 per cent solution of phloroglucinol in ethanol (Borenfreund and Dische, 1957). After spraying with the reagent, the paper was dried at room temperature and then placed for 2 minutes in an electric oven adjusted at 90°C. In the case of phenol detection, the chromatogram was sprayed either with 2 per cent ethanolic solution of ferric chloride (Svendson, 1951) or 1 per cent sodium nitrite dissolved in

10 per cent acetic acid and then with N sodium hydroxide solution and air dried (Roberts and Wood, 1953).

Silicated epidermal cells of the bud scales:

The outer scales from 20 lateral eye-buds from the middle portion of the canes of each variety were carefully removed by means of a pair of forceps and placed in a test tube. These were then macerated by the Schultz's method as reported by Adyanthaya and Rangaswami (1952). Concentrated nitric acid was poured into the test tube to cover the bud scales and a few crystals of potassium chlorate were added. The mixture was then gently heated until bubbles were evolved and the reagents reacted to make the material white. The contents of the tube were then poured into a dish of cold water and thoroughly washed. The peels were selected and stained with 0.5 per cent safranin. Counts of the silicated cells were made under the high power of the microscope. Twenty microscopic fields were counted for each variety and the average number of silicated epidermal cells per unit area was calculated.

Continuity of vascular bundles from the stalk to the leaf-sheath:

After bringing the sugarcane plants from the field, their top portions consisting of few internodes with foliage were cut under water so that transpiration current was not broken. Thecut-ends were then immersed in dilute India-ink solution (1 part India-ink and 3 parts water). Care was taken to see that the level of the ink solution after dipping

the canes did not reach the node above the cut-end. After keeping in the ink solution for 24 hours at room temperature, the canes were removed and the basal ½ cm. portion of the leaf-sheath above the cut-end which was dipped in the ink solution was cut out by means of a sharp knife. This was then examined under a dissecting microscope. The total number of vascular bundles and the ones showing the presence of the ink were counted. The percentage of ink-showing vascular budnles in the leaf-sheaths of each variety was then calculated, by examining the leaf-sheath spread out, under the dissecting microscope.

Phenol extraction:

The tissue of the 8th node from top (from ½ cm. below the point of attachment of the leaf-sheath upto the growth ring), the leaf-sheath attached to the 8th node (10 cm. portion from the base) and the internode between the 8th and 9th node (4 cm. portion from the middle) of 5-6 canes selected at random from each variety in the month of August, were separately cut into small pieces by means of a sharp knife. Ten gram samples of the cut-pieces from each lot were then weighed out and immediately placed, separately, in 100 ml. Erlenmeyer flasks, each containing 50 ml. of 95 per cent ethanol and boiled in a water bath for 3-5 minutes in order to destroy the polyphenol oxidase activity. The samples were then cooled, the extract decanted into separate flasks and

the plant material was crushed well with pestle and mortar. The crushed material was then added to the extract and the mixture allowed to stand overnight after which it was filtered through Whatman No. 42 filter paper. The residue was re-extracted twice with 25 ml. of 80 per cent ethanol, each time allowing it to stand for 3 hours after the addition of ethanol. The filtrates from the respective tissues of each variety were combined and the volume made upto 100 ml. with 50 per cent ethanol. The extracts were stored in a refrigerator for further experiments.

Estimation of phenols:

The phenol content of different varieties of sugarcane was estimated in chlorogenic acid equivlents by the colorimetric method by adopting the Hoepfner-Vorsatz test described by Reeve (1951).

To 10 ml. of the alcohol extract was added 40 ml. of distilled water to form a five-fold dilution. To 5 ml. of this were added 1 ml. of 10 per cent freshly prepared sodium nitrite, 1 ml. of 20 per cent urea, 1 ml. of 10 per cent acetic acid and after 3 minutes 2 ml. of 2 N sodium hydroxide. The optical density of the solution was then measured in a Lumetron colorimeter (model No. 401 A) using a blue 420 m μ filter. The concentration of phenols in the solution was read directly from a standard curve constructed by using the optical densities of different concentrations of pure chlorogenic acid treated with the above reagents in the same procedure. The

concentration of phenols in mg./100 gm. of the tissue was then calculated.

Extraction of amino acids from the nodal regions of sugarcane:

The 8th node from top of 5 canes in each variety (from ½ cm. below the point of attachment of the leaf-sheath upto the growth ring) selected at random, in the month of August, were cut into small pieces and 10 gram samples from each lot were used for the extraction of amino acids. The procedure adoptedwas essentially the same as that already described for phenol extraction, except that only 70 per cent ethanol was used in this case and the tissues were boiled in a water-bath for one hour for the first extraction and 30 minutes for each subsequent extraction. A long condensor tube was fitted at themouth of the flask in order to avoid the loss of alcohol during boiling. The extracts of each variety were combined and the volume made upto 100 ml. with 50 per cent ethanol. These were then stored in a refrigerator for further experiments.

Estimation of free amino acids:

Free amino acids were determined in glycine equivalents, by thecolorimetric method described by Wiggins and Williams (1955).

Reagents:

(1) Ninhydrin solution:- Ninhydrin 1.0 gm. dissolved in 100 ml. of isopropyl alcohol. The solution was stored in a brown glass bottle.

(ii) Acetate buffer:- Sodium hydroxide 40 gm. and glacial acetic acid 100 ml. dissolved separately in distilled water, then mixed together and volume made upto one litre.

(iii) Aqueous isopropyl alcohol:- Equal volumes of isopropyl alcohol and distilled water.

(iv) Clarifying mixture:- Bentonite clay 15 gm., kaolin 20 gm. and acid washed activated charcoal 50 gm., suspended in one litre of distilled water.

Procedure:

The solution for analysis was prepared by mixing 20 ml. of the alcohol extract with 5 ml. of clarifying solution and diluting to 100 ml. with distilled water. It was then filtered through Whatman No. 42 filter paper, which gave a colourless filtrate representing a five-fold diluted extract.

The filtrate 10 ml., ninhydrin solution 2.5ml. and acetate buffer 2.5 ml., were pipetted into a 50 ml. graduated flask which was then suspended in a bath of briskly boiling water for 30 minutes. Two determinations were made for each sample simultaneously. At the end of the heating period the flasks were removed, cooled at room temperature and the contents made upto 50 ml. with aqueous isopropanol and mixed well by shaking. The optical density of the solution was measured in a Lumetron colorimeter, using a 420 m μ filter.

The concentration of amino acids in the solution was read directly from the standard curve constructed by using the optical densities of different concentrations of glycine, treated according to the above procedure. The concentration of amino acids in mg./100 gm. of the nodal region was then calculated.

Any other technique if used during the investigation will be described under the respective experimental portion.

IV. EXPERIMENTAL RESULTS

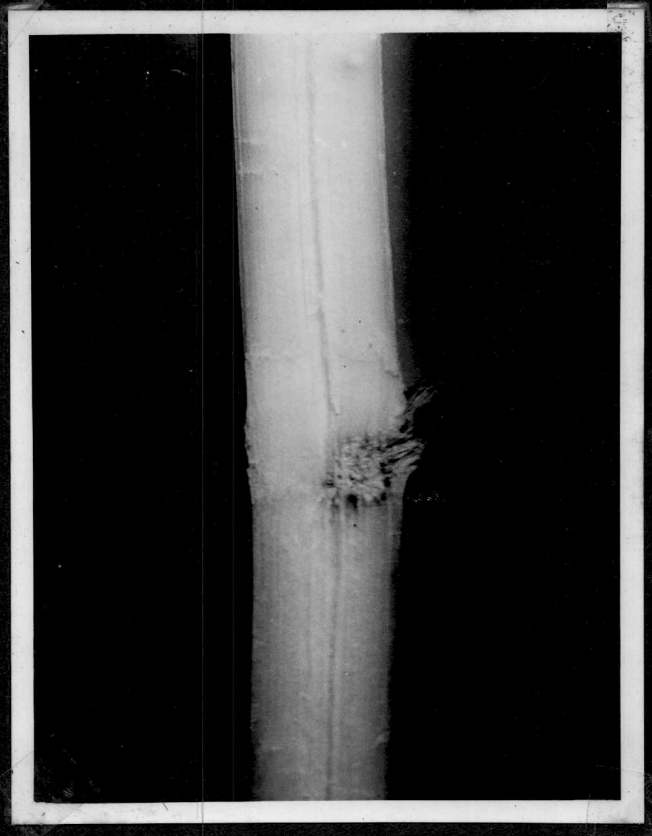
Varietal reaction to red rot infection through the nodal regions of sugarcane:

It has been conclusively proved that considerable amount of red rot infection takes place through the nodal regions at the upper and middle portions of standing canes in the field, in the entire absence of basal infection or borer holes (Chona and Padwick, 1942). The spores of Colletotrichum falcatum produced on the leaf midrib lesions get washed down with dew or rain in the cavity formed between the stalk and leaf-sheath, thus reaching the nodal regions and causing infection. Nodal infection studies conducted by Chona (1950) revealed that the percentage of infection through the nodal region was maximum when the plants were artificially inoculated in the month of August. The standing canes of 6 varieties of sugarcane were inoculated at the nodal region in the month of August, as described in "Materials and Methods". In the case of controls, only 2 ml. of sterile water was poured into the cavity of the leaf-sheath. The plants were irrigated liberally after inoculation. Final observations were taken in the month of October (about 2 months after inoculation). The canes were examined for red rot infection by gently scraping the rind of the inoculated nodes and also by splitting open the canes. The results obtained are presented in Table I. Fig. 1-3 show red rot infection through the nodal regions.

Fig. 1. Red rot infection at the nodal region of sugarcane originating from the root primordia.

Fig. 2. Red rot infection originating from the leaf-scar region (the point of attachment of the leaf-sheath to the node) of sugarcane stalk.

Fig. 3. Red rot infection originating from the leaf-scar region and spreading to the lateral bud tissues.



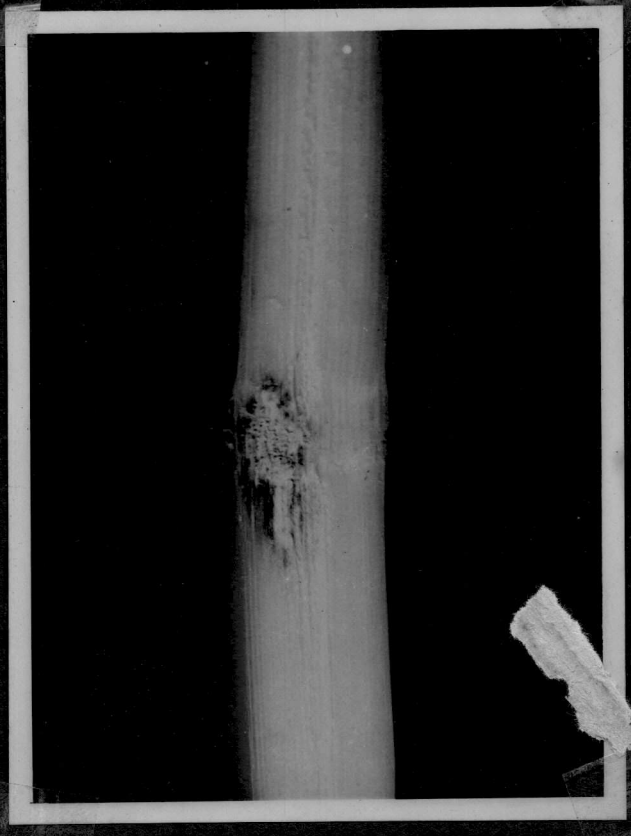


TABLE I. Percentage of red rot infection through the nodal regions of artificially inoculated sugarcane varieties.

Cane variety	Number of inoculated canes examined	No. showing infection	% infection
Co. 445	50	30	60.0
Co. 331	40	19	47.5
Co. 312	40	15	37.5
Co.1181	40	16	40.0
Co.1070	40	9	22.5
Co. 285	25	3	12.0

It would be observed from Table I that though the fungus could enter through the nodal regions of all the varieties tested, the percentage of infection varied considerably in different varieties. Variety Co. 445 developed the greatest amount of infection, while Co. 285 showed the least infection indicating that it offered greater resistance to nodal infection. Co. 1070 also showed considerable amount of resistance to nodal infection. No infection was observed in the controls. In most of the cases, it was noticed that the infection originated at the point of attachment of the leaf-sheath to the node. In some cases, however, the infection started from the root primordia and in a few cases through the eye-buds.

Isolation of Colletotrichum falcatum from the nodes of apparently healthy stalks of sugarcane:

The nodal tissues of apparently healthy stalks of sugarcane have been shown to harbour the fungus, Colletotrichum falcatum, in a latent form. Such plants pass undetected at the time of selection of setts for planting in the succeeding season. This helps in the perpetuation of the fungus and accumulation of the inoculum which may lead to the outbreak of the disease in an epiphytotic form under favourable environmental conditions. The incipient red rot infection at the nodal regions was, therefore, studied by isolating the fungus from the nodes of apparently healthy sugarcane plants in the months of September and December. The nodes (from the leaf-scar region upto the growth ring), from 15-20 canes of each variety, selected at random, were cut out by means of a sharp knife, after carefully removing the leaf-sheaths. About 100-120 nodes were studied from each variety. Each node was cut into 4 pieces, surface sterilized for 3-5 minutes with 1:1000 mercuric chloride solution, and then washed with three changes of sterilized water and placed in sterile Petri-dishes containing oat-meal-agar medium. All the four pieces from one node were placed in one Petri-dish. The plates were incubated at 25-27°C and observations for the presence of the fungus were made after 6-8 days. If the fungus appeared from one or more pieces of the node, the node was considered to be infected. The results obtained are presented in Table II.

TABLE II. Percentage of nodes giving the fungus, Colletotrichum falcatum from apparently healthy stalks of sugarcane

Cane variety	Percentage of nodes yielding the fungus	
	September	December
Co. 445	4.1	8.0
Co. 331	6.0	3.0
Co. 312	6.6	12.0
Co.1181	3.0	1.0
Co.1070	2.0	0.0

It would be observed from the data in Table II that the fungus persisted in a latent form in the nodal regions of apparently healthy stalks of sugarcane. However, the percentage of nodes yielding the fungus on isolation is not very high under the conditions of the experiment.

Isolation of Colletotrichum falcatum from the leaf-sheaths of sugarcane:

It has been reported by Steib and Chilton (1951) that Colletotrichum falcatum first developed on the leaf-sheath and finally infected the nodal tissues of sugarcane. The extent of natural infection of the leaf-sheaths by the conidia washed down from the leaf midrib lesions was studied by isolating the fungus in the month of December. The 10th, 11th and 12th leaf-sheaths (from top) were selected from each

cane. Four small pieces (about ¼ cm. square) from the bottom 10 cm. portion of each leaf-sheath were used for isolation. The leaf-sheath bits were surface sterilized for 2-3 minutes with 1:1000 mercuric chloride solution, washed with three changes of sterile water and plated on oat-meal-agar medium. All the 4 bits from one leaf-sheath were placed in one Petri-dish. Observations were taken after 7 days' incubation at 25-27°C. If one or more pieces from a leaf-sheath yielded the fungus, the leaf-sheath was considered to be infected. The results obtained are presented in Table III.

TABLE III. Percentage of sugarcane leaf-sheaths yielding the fungus, Colletotrichum falcatum

Cane variety	Number of leaf-sheaths plated	No. yielding the fungus	Percentage of leaf-sheaths yielding the fungus
Co. 445	60	8	13.3
Co. 331	60	7	11.6
Co. 312	60	7	11.6
Co.1161	60	6	10.0
Co.1070	60	5	8.3

The data presented in Table III clearly indicate that the fungus develops on the leaf-sheaths of sugarcane. Since all the tissues from the leaf-sheaths and all the leaf-sheaths from one plant were not included for the isolation, the actual percentage of infection was probably higher than what

has been obtained, as similar infection may possibly be present in the other leaf-sheaths of the plant also.

Effect of leaf-sheath water on the spore germination of Colletotrichum falcatum:

The exudates from different plant structures have been reported to stimulate the spore germination of several pathogenic microorganisms, thus aiding in the infection of their respective host plants. In a number of cases the quality of exudate could be correlated with the resistance or susceptibility of the plant to the invading microorganism. Mayeaux and Colmer (1960) demonstrated the presence of varying amounts of carbohydrates in the leaf-sheath water of sugarcane. The effect of leaf-sheath water from different sugarcane varieties on the spore germination of C. falcatum was, therefore, studied as per method described under "Materials and Methods". Observations were taken after 16 hours' incubation at 25-27°C. The results obtained are presented in Table IV.

It will be observed from Table IV that the leaf-sheath water from different sugarcane varieties markedly stimulated the spore germination of C. falcatum. However, there was no appreciable difference between the 6 varieties tested with regard to the percentage of spore germination. As regards the length of the germ tubes, it was noticed that the germ tubes produced in the leaf-sheath water were very long, about 3-4 times the length of the spore, and

TABLE IV. Spore germination of Colletotrichum falcatum in the leaf-sheath water of different sugarcane varieties

Cane variety	Per cent spore germination	
	August	September
Co. 445	78.0	76.8
Co. 331	80.7	75.1
Co. 312	78.9	75.8
Co.1181	73.7	70.8
Co.1070	75.2	76.2
Co. 285	76.6	73.5
Control (Distilled water)	38.6	36.4

that about 25 per cent of the germ tubes produced appressoria at their ends. The germ tubes produced in distilled water were small and most of them produced appressoria.

Chromatographic analysis of the leaf-sheath water for the detection of sugars and amino acids:

As the leaf-sheath water stimulated the spore germination of C. falcatum, an attempt was made to detect the nutrients present in the same. Twenty ml. of the leaf-sheath water collected from Co. 331 was concentrated to 2 ml., approximately, over a steam-bath and spotted on Whatman No. 1 filter paper. The details of chromatographic

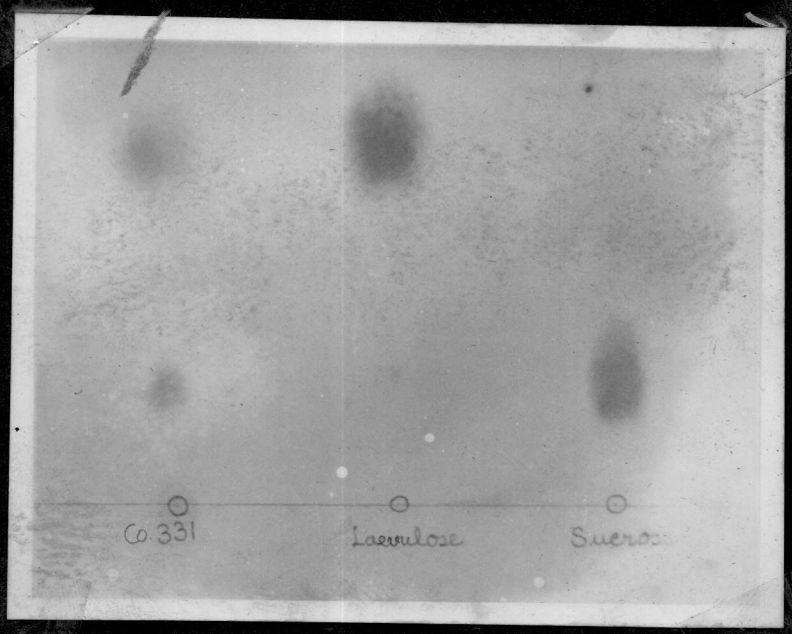
analysis have been given in Chapter III. On spraying with phloroglucinol solution and drying in the oven, two dark brown spots having Rf. values 0.13 and 0.24 appeared on the paper. These Rf. values were compared with the Rf. values of different sugars calculated from standard chromatograms prepared by using pure sugars and were found to be sucrose and laevulose. Pure sucrose and laevulose were also spotted on another paper alongwith the sample and chromatogram prepared, in order to confirm their presence in the leaf-sheath water (Fig. 4).

On spraying with ninhydrin solution and heating in the oven for the detection of amino acids, no spots developed on the paper showing thereby that there was no amino acid present in the leaf-sheath water.

Continuity of vascular bundles from the stalk to the leaf-sheath of sugarcane varieties:

Some investigators have reported that the resistance of sugarcane varieties could be correlated with the number of continuous vascular bundles in the stalk: the resistant varieties generally having lesser number of continuous vascular bundles than the susceptible ones. The percentage of vasculer bundles continuous from the stalk to the leaf-sheath of different sugarcane varieties was studied with a view to find out whether the number of continuous vascular bundles could be correlated with the resistance or susceptibility to red rot infection through the nodal region. The

Fig. 4. Chromatogram showing the presence of sucrose and laevulose in the leaf-sheath water collected from sugarcane variety Co. 331.


Co. 331
Laevulose
Sucrose

method adopted for the study has been described in Chapter III. Ten to fifteen canes from each of the 6 varieties were used for this study. The percentage of ink-stained vascular bundles in the varieties studied is given in Table V.

TABLE V. Percentage of vascular bundles continuous from the stalk to the leaf-sheath of sugarcane varieties.

Cane variety	Average number of		Percentage of continuous vascular bundles
	total vascular bundles	continuous vascular bundles	
Co. 445	90	30	33.3
Co. 331	95	33	34.7
Co. 312	88	32	36.4
Co.1181	100	38	38.0
Co.1070	78	27	34.6
Co. 285	76	26	34.2

It will be noticed from Table V that both the resistant and susceptible varieties have more or less the same percentage of vascular bundles continuous from the stalk to the leaf-sheath and that no correlation existed between the percentage of the continuous vascular bundles and the resistance or susceptibility to nodal infection.

Silicated epidermal cells in the bud scales of sugarcane varieties:

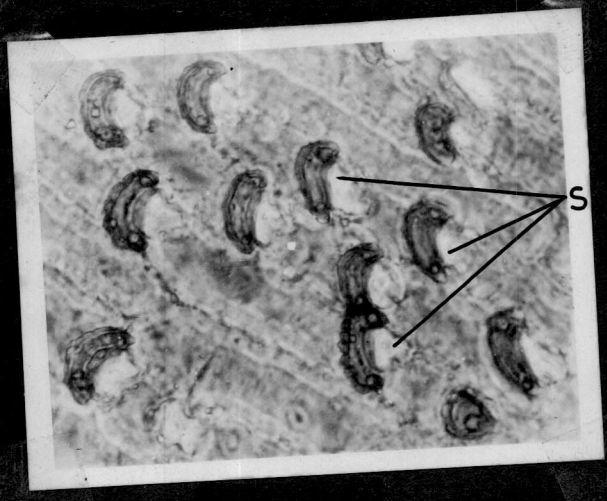
Leaves of rice plants resistant to blast and blight disease have been reported to possess more number of silicated epidermal cells than the susceptible ones. The silicated epidermal cells of the bud-scales of 6 different sugarcane varieties were studied in order to find out whether there was any difference in the number of silicated cells in the susceptible and resistant varieties. The epidermal peelings of the bud scales after staining with safranin were observed under the microscope. Only the non-silicated cells took the stain while the silicated cells remained clear and unstained (Fig. 5). The data are recorded in Table VI.

TABLE VI. Average number of silicated epidermal cells in the bud scales of sugarcane varieties

Cane variety	Silicated epidermal cells per microscopic field
Co. 445	32
Co. 331	34
Co. 312	33
Co.1181	36
Co.1070	33
Co. 285	34

Fig. 5. Silicated epidermal cells in the bud scale of sugarcane variety Co. 331.

S = Silicated epidermal cells.


S

The results presented in Table VI clearly show that there is no correlation between the silicated epidermal cells of the bud scales and the resistance or susceptibility to the infection by C. falcatum, since the average number of silicated epidermal cells was more or less the same in the six varieties studied, which possess greatly different degree of resistance to red rot.

Effect of the extract from the nodal regions of sugarcane varieties on the spore germination and growth of C. falcatum:

The nodal extract was prepared in the following manner:

Twenty five grams of the nodal tissue from the middle portions of 4 cane varieties, collected in the month of September, were separately weighed, cut into small pieces and boiled for one hour in 250 ml. of distilled water. After cooling, the extracts were separately filtered through muslin cloth and the volume made upto 250 ml. with distilled water. Twenty ml. aliquots of the extracts were pipetted into 100 ml. Pyrex flasks, which were then plugged with cotton wool and sterilized in an autoclave at 15 lb. pressure for 20 minutes. These were used for the following experiments:

(a) Spore germination of C. falcatum in the nodal extracts:

Standard spore suspensions of the fungus were prepared in the nodal extracts and the percentage of spore germination was studied as described in Chapter III.

Observations were taken after 16 hours' incubation at 25-27°C and the results obtained are presented in Table VII.

TABLE VII. Spore germination of C. falcatum in the nodal extracts of sugarcane varieties

Cane variety	Per cent spore germination
Co. 445	82.6
Co. 331	80.3
Co.1070	80.6
Co. 285	79.9
Control (Distilled water)	40.1

It would be noticed from the data in Table VII that there is practically no difference in the percentage of spore germination of the fungus in the nodal extracts of different cane varieties tested. It was noticed that the germ tubes produced in the nodal extracts were comparatively longer than those produced in distilled water. Moreover, most of the germ tubes produced in distilled water had formed appressoria, while only about 25 per cent of the germ tubes produced in the nodal extracts formed appressoria at their ends.

(b) Growth of C. falcatum in the nodal extracts:

Flasks containing the nodal extracts (20 ml. each) of the 4 sugarcane varieties were inoculated with a

standard spore suspension of the fungus (having 20-25 spores per microscopic field under the low power), prepared in sterilized water. Half ml. of the spore suspension was poured into each flask, by means of a sterile pipette. Three flasks for each variety were inoculated. The flasks were incubated at 25-27°C. After 12 days' growth of the fungus, the mycelial mat from each flask was removed and separately washed with distilled water on previously dried and weighed Whatman No. 42 filter papers. These were then dried in an oven adjusted at 60°C for 72 hours, cooled in a desiccator and weighed in an analytical balance. The weight of the filter paper was substracted in each case and the average mycelial weight for three flasks in each variety was calculated. The results obtained are presented in Table VIII.

TABLE VIII. Mycelial weight of Colletotrichum falcatum after 12 days growth in the nodal extracts of sugarcane varieties

Cane variety	Weight of mycelium in milligrams (dry weight basis)
Co. 445	112.0
Co. 331	106.5
Co.1070	105.3
Co. 285	108.3

The results presented in Table VIII show that the growth of mycelium was almost the same in the nodal extracts of all the 4 varieties.

(c) Growth of *C. falcatum* in the nodal extract agar:

Two per cent agar was added to the nodal extracts of the 4 cane varieties and mixed well by boiling. These were then poured into separate 100 ml. flasks, each having 20 ml. of the medium. The flasks were plugged with cotton wool and sterilized at 15 lb. pressure for 20 minutes. When sufficiently cooled, the nodal extract agar from each flask was poured into separate, sterile Petri-dishes and allowed to solidify. Four plates were poured for each variety. One loopful of a standard spore suspension of the fungus (having 20-25 spores per microscopic field under low power) was placed in the centre of the medium in each Petri-dish, by means of a sterilized inoculating loop. The plates were incubated at 25-27°C. Observations were taken at the end of 6 days. The diameter of the colony was measured and the average of 4 plates in each variety was calculated. The results obtained are recorded in Table IX.

TABLE IX. Growth of *C. falcatum* on the nodal extract agar of sugarcane varieties.

Cane variety	Diameter of colony (mm)	Sporulation
Co. 445	78.5	Moderate
Co. 331	77.2	"
Co.1070	77.6	"
Co. 285	76.8	"

It would be observed from Table IX that there was no difference in the growth of the fungus in the nodal extract agar of the 4 varieties studied, with regard to the colony diameter or sporulation. The mycelium was fluffy and greyish-white in colour in all the cases.

Effect of the leachate from the nodal region of sugarcane varieties on the spore germination of C. falcatum:

The nodes from the middle portion of 3 canes from each variety were separately cut into thin slices in the month of September. Five gram samples were weighed from each lot and placed in separate specimen tubes and 15 ml. of distilled water were added to each tube. The tubes were then corked and placed in the freezing chamber of a refrigerator for 48 hours. The leachates were separately filtered through muslin cloth and their effect on spore germination of C. falcatum was studied as described in Chapter III. Observations were taken after 16 hours' incubation at 25-27°C. The results obtained are presented in Table X.

It will be noticed from Table X that the spore germination of the fungus was more or less the same in the nodal leachates of all the 4 varieties studied. The length of germ tubes produced in the leachates of all the varieties was longer than that produced in distilled water.

TABLE X. Spore germination of C. falcatum in the nodal leachates of sugarcane varieties.

Cane variety	Per cent spore germination
Co. 445	83.6
Co. 331	84.9
Co.1070	81.9
Co.285	84.1
Control (Distilled water)	32.3

Free amino acid content of the nodal region of sugarcane varieties:

In a number of cases the amino acid content of the plant has been correlated with the susceptibility or resistance to parasitic diseases. Susceptible plants have been generally reported to contain more amino nitrogen in their tissues than the resistant ones.

The free amino acid content in the nodal region of sugarcane varieties susceptible and moderately resistant to red rot infection through the nodal region was, therefore, studied. The method adopted has been described in Chapter III. The amino acid content of the nodal region was calculated in glycine equivalents from the standard curve (Fig. 6) prepared by using the optical densities of different concentrations of glycine. The results obtained are given in Table XI.

STANDARD CURVE FOR THE DETERMINATION OF FREE AMINO ACID CONTENT OF SUGARCANE (IN GLYCINE EQUIVALENTS)

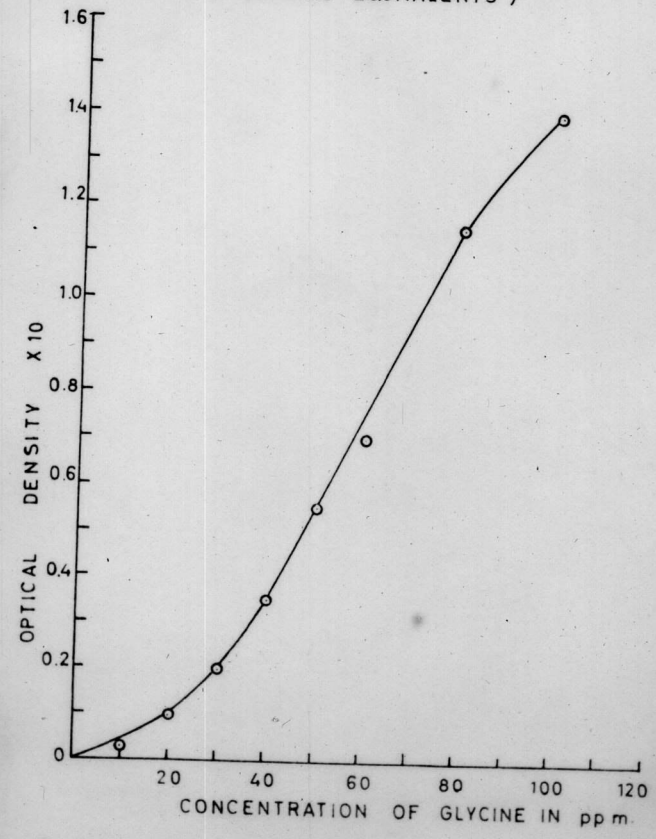


TABLE XI. Free amino acid content in the nodal region of sugarcane varieties

Cane variety	Free amino acid content*
Co. 445	21.50
Co. 331	20.25
Co. 1070	18.00
Co. 285	15.75

*In mg./100 gm. fresh weight.

It will be seen from Table XI that the amino acid contents in the nodal region of the red rot susceptible sugarcane varieties are more than that of the moderately resistant ones. Cane variety Co. 445 which is highly susceptible to red rot infection has the maximum amino nitrogen in the nodal region, while variety Co. 285 which is comparatively resistant has the least, indicating thereby that the quantity of amino nitrogen in the nodal region can be correlated with the susceptibility or resistance of a variety to nodal infection. However, a very large number of cane varieties of known resistence or susceptibility to red rot must be tested to draw a general conclusion to establish such correlation.

Chromatographic detection of phenols in the nodal region of sugarcane varieties:

Sugarcane varieties resistant to red rot disease have been reported to contain more phenols than the susceptible ones (Abbott, 1938 and Parthasarathy and Vijayasarathy, 1958). Phenols like chlorogenic acid, caffeic acid, catechol and protocatechuic acid have been shown to offer resistance to parasitic diseases in certain crop plants. An attempt was, therefore, made to detect the phenols in sugarcane stalks, with a view to study whether they played any role in offering resistance to red rot.

Fifty ml. of the phenol extracts from the nodal region of 4 sugarcane varieties (Co. 445, Co. 331, Co. 1070 and Co. 285) were concentrated to dryness, in separate crucibles placed in an oven adjusted at 60°C. The residue was redissolved in 2 ml. of 70 per cent ethanol, added to each crucible. The samples were then spotted on Whatman No. 1 filter paper and chromatograms prepared as described in Chapter III. Standard chromatograms, using pure chlorogenic acid, caffeic acid and catechol were also prepared in the same procedure and their Rf. values calculated. After allowing the developing solvent to ascend for 18 hours, the papers were removed from the chamber and air-dried. On spraying with 2 per cent ethanolic solution of ferric chloride, a dark-green coloured spot having the Rf. value 0.6 appeared on the paper. This corresponded with pure

chlorogenic acid having the Rf. value 0.62, which was calculated from the standard chromatogram. No spot corresponding to caffeic acid or catechol was obtained. Pure chlorogenic acid was also co-chromatographed with the samples in order to confirm its presence in the nodal extracts (Fig. 7). The slight difference between the Rf. values of pure chlorogenic acid and that obtained from the nodal extracts, could be due to the interfering substances in the samples, as reported by Dinkel (1963). On spraying with 1 per cent sodium nitrite in 10 per cent acetic acid followed with N NaOH solution, the spot corresponding to chlorogenic acid appeared reddish-brown in colour. This reaction, according to Roberts and Wood (1951), is specific to chlorogenic acid.

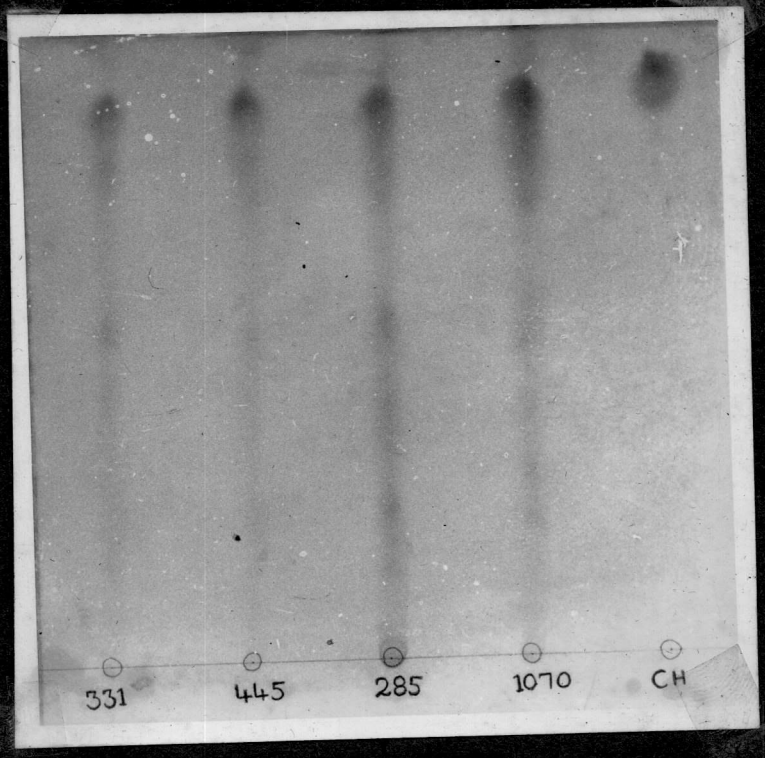
It was observed that chlorogenic acid was present in the nodal extracts of all the 4 sugarcane varieties. No attempt was made to determine the quantitative difference in the chlorogenic acid content of the different varieties, chromatographically.

Effect of different concentrations of chlorogenic acid in distilled water, on the spore germination of *C. falcatum*:

Pure chlorogenic acid was dissolved in sterile distilled water to have various concentrations ranging from 50 - 2000 ppm. Standard spore suspensions were prepared in these chlorogenic acid solutions and germination studies

Fig. 7. Chromatogram showing the presence of chlorogenic acid in the nodal region of sugarcane varieties.

CH = pure chlorogenic acid.


331
445
285
1070
CH

were conducted as described in Chapter III. In this experiment, the spores after centrifugation with distilled water were washed with 50 ppm chlorogenic acid solution as described by McLean et al. (1961). The suspension was then centrifuged at 2000 rpm for 20 minutes and the supernatant poured off. Observations on germination were taken at the end of 16 hours' incubation at 25-27°C. The results obtained are given in Table XII.

TABLE XII. Spore germination of C. falcatum in various concentrations of chlorogenic acid in distilled water

Concentration of chlorogenic acid	Per cent spore germination
0	42.7
50	80.4
125	64.0
250	50.6
500	27.5
1000	4.2
2000	0.0

It would be observed from Table XII that chlorogenic acid at higher concentrations has an inhibitory effect on the spore germination of C. falcatum, there being complete inhibition at 2000 ppm. At lower concentrations, however,

it stimulated the spore germination, the maximum being at 50 ppm., and gradually decreased as the concentration increased. Moreover, at lower concentrations, (from 50 - 250 ppm.) the germ tubes produced were very long with fewer number of appressoria, while the germ tubes produced in higher concentrations of chlorogenic acid and also in distilled water were much shorter and most of them formed appressoria at their ends.

Effect of different concentrations of chlorogenic acid added to the nodal leachate of sugarcane, on the spore germination of C. falcatum:

To the nodal leachate of sugarcane variety Co. 445, prepared as described earlier in this Chapter, different concentrations of chlorogenic acid was added in order to study its effect on the spore germination of the fungus. Equal quantities of chlorogenic acid solutions and the nodal leachate were mixed together so as to have concentrations ranging from 125 to 1000 ppm. of chlorogenic acid. Standard spore suspension was prepared in each concentration and the spore germination studied. Observations were taken after 16 hours' incubation at 25-27°C. The results obtained are presented in Table XIII.

It is evident from Table XIII that the spore germination of the fungus was not at all affected by lower concentrations of chlorogenic acid in the nodal leachates

TABLE XIII. Spore germination of C. falcatum in the nodal leachate of cane variety Co. 445 having different concentrations of chlorogenic acid.

Concentration of chlorogenic acid	Per cent spore germination
125	84.6
250	82.2
500	79.4
1000	73.7
Control I (Leachate only)	83.6
Control II (Distilled water)	39.7

of cane variety Co. 445. At 1000 ppm. concentration, however, there was about 10 per cent reduction in spore germination, when compared with that in Control-I (leachate). The length of germ tubes was very long in all the treatments as well as in Control-I, and only a few appressoria were formed. But the germ tubes produced in Control-II (Distilled water) were very short and most of them formed appressoria at their ends.

Phenolic content of sugarcane varieties:

The phenolic content of 4 sugarcane varieties was determined by adopting the Hoepfner-Vorsatz test, as described

in Chapter III. This test has been found to be useful in differentiating a number of phenols in solution (Reeve, 1951).

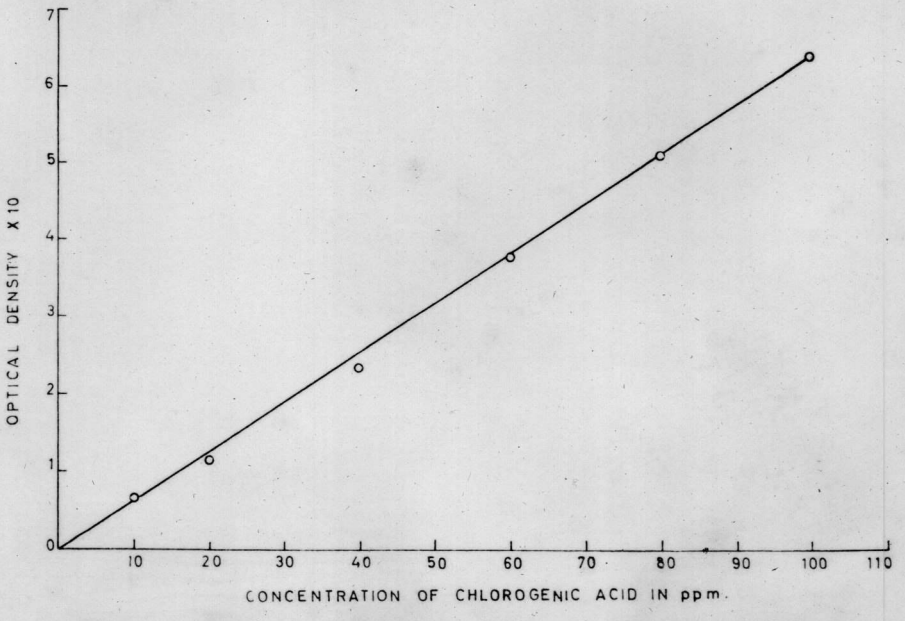
On adding the reagents, pure chlorogenic acid as well as the extracts from the sugarcane tissues developed a cherry-red colour. The intensity of the colour developed was measured by means of a Lumetron colorimeter and the amount of phenol (mg./100 gm. of the tissue) was calculated in chlorogenic acid equivalents, from the standard graph (Fig. 8) prepared by using different concentrations of pure chlorogenic acid in distilled water.

It has been reported by Reeve (1951) that catechol gives the same colour as that of chlorogenic acid, when tested by the above method. Since the nodal extracts of sugarcane, analysed by the chromatographic method, did not show the presence of catechol, it is assumed that the cherry-red colour reaction of the sugarcane extracts on applying the test, is mainly due to the presence of chlorogenic acid in the same.

The phenolic content of the node, internode and leaf-sheath of 4 sugarcane varieties, expressed in chlorogenic acid equivalents are given in Table XIV and illustrated in Fig. 9.

The results in Table XIV clearly show that the sugarcane varieties moderately resistant to red rot infection have a higher phenolic content in their tissues, than the susceptible ones, the differences being more pronounced in

STANDARD CURVE FOR THE DETERMINATION OF PHENOL CONTENT IN SUGARCANE
(IN CHLOROGENIC ACID EQUIVALENTS)



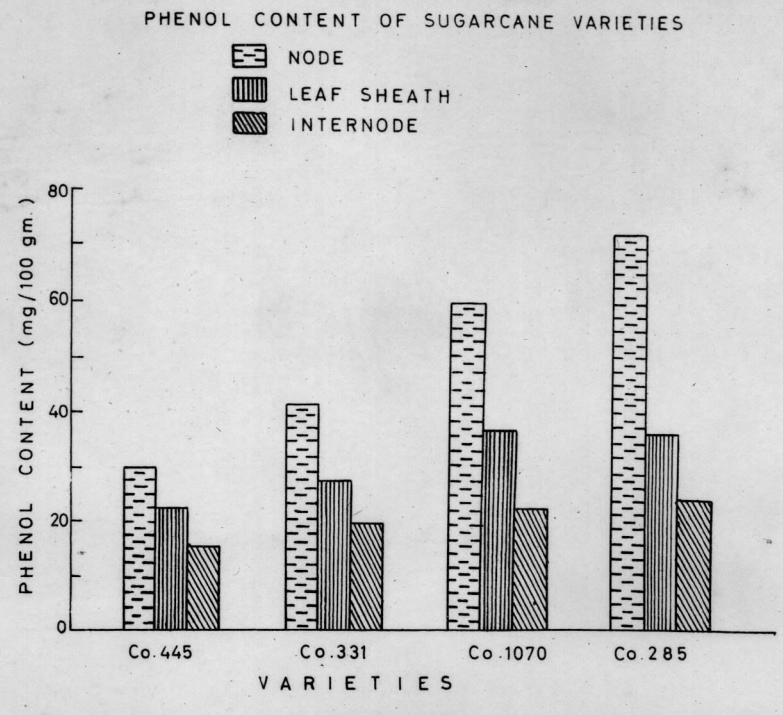

PHENOL CONTENT OF SUGARCANE VARIETIES
NODE
LEAF SHEATH
INTERNODE
PHENOL CONTENT (mg/100 gm.)
80
60
40
20
0
Co.445
Co.331
Co.1070
Co.285
VARIETIES

TABLE XIV. Phenol content of sugarcane varieties

Cane variety	Phenol content (mg./100 gm. fresh weight)		
	Node	Internode	Leaf-sheath
Co. 445	30.0	15.5	22.5
Co. 331	41.0	19.0	27.0
Co.1070	59.0	22.0	36.0
Co. 285	70.5	24.0	36.0

the nodes. The phenol content of all the three parts (node, internode and leaf-sheath) of the moderately resistant varieties is more than that in the susceptible ones. Out of the 4 varieties tested, a moderately resistant variety, Co. 285, has the maximum phenol content, while a highly susceptible variety, Co. 445, has the least. The nodes of all the varieties tested contained the maximum amount of phenol, while the internodes contained the least.

Phenol content of sugarcane stem pices inoculated with Colletotrichum falcatum:

It has been reported by Kuc et al. (1956) that inoculation of potato slices, with Helminthosporium carbonum resulted in the production of chlorogenic and caffeic acids in them. The phenol content of the cut pieces of sugarcane stem after inoculation with Colletotrichum falcatum was

studied as described below:

The 8th node from top, with one internode each on both the sides, from 8-10 canes, selected at random from each variety, was cut and brought to the laboratory in the month of August and the leaf-sheath attached to the node was removed by cutting at the base. The stem pieces were cleaned with cotton wool, surface sterilized with rectified spirit and then washed with sterilized water. Slices of the nodal region as also the internodes (approximately 1 cm. thick) from each variety were separately cut by means of a sterile knife and placed in sterilized Petri-dishes lined with moist, sterile filter paper. The slices were then inoculated with a standard spore suspension (having 25-30 spores per microscopic field under low power) of *C. falcatum*. Three to four drops of the spore suspension were poured over each slice, by means of a sterile pipette. In the case of controls, only 3-4 drops of sterilized distilled water were poured over each slice. The plates were then incubated at 25-27°C for 72 hours. After the incubation, 10 gram samples of the nodal as well as internodal tissues from each variety (Inoculated as well as Control) were separately weighed and the phenol extracted as described in Chapter III. The phenol content of the inoculated as well as Controls in each variety was then calculated in chlorogenic acid equivalents as described before. The results obtained are presented in Table XV.

TABLE XV. Phenol content of sugarcane stem pieces inoculated with *Colletotrichum falcatum*

Cane variety	Cane portion used	Phenol content*		Increase in phenol content
		Control	Inoculated	
Co. 445	Node	21.5	23.0	1.5
	Internode	12.0	12.0	0.0
Co. 331	Node	35.0	37.0	2.0
	Internode	17.5	18.5	1.0
Co. 1070	Node	54.0	55.5	1.5
	Internode	21.0	22.5	1.5
Co. 285	Node	62.0	63.5	1.5
	Internode	22.5	23.5	1.0

*In mg./100 gm. fresh weight.

It would be seen from Table XV that the phenol content of the stem pieces of sugarcane varieties, susceptible, as well as moderately resistant to red rot infection, increased in response to inoculation with C. falcatum. However, the increase in phenol content is only very slight when compared to the initial phenolic content in the stem tissues.

Phenol content in the stem tissues of growing canes artificially inoculated with Colletotrichum falcatum:

The effect of artificial inoculation with C. falcatum on the phenol content of growing sugarcane stem tissues was studied as described below:

Ten to twelve canes selected at random from each variety were inoculated with C. falcatum in the month of August, by the standard Plug Method described by Chona (1954). The upper portion of the 9th internode from top of each cane was surface sterilized by wiping with a swab of cotton wool dipped in rectified spirit and a hole (1 cm. below the base of the 8th leaf-sheath from top) having depth upto about two-third the thickness of the cane was made by means of a sterile steel cork-borer. Three drops of standard spore suspension of the fungus were poured into the hole by means of a sterile pipette and the plug replaced in the cavity. The point of inoculation was sealed with white wax having a low melting point, wrapped with alkathene foil and firmly tied with string to avoid extraneous infection.

Seven days after inoculation, 4 canes each from the inoculated as well as the uninoculated controls of each variety were cut and brought to the laboratory. The inoculated nodes and internodes were separately cut out from the canes of each variety and the phenol extracted as described in Chapter III. The phenol extraction from the corresponding tissues of the healthy canes was also done separately. In the case of inoculated internode, a 4 cm. portion (1 cm. away from the plug) was used for extraction. The phenol contents of the various tissues were estimated as described before. The results obtained are presented in Table XVI and illustrated in Fig. 10.

The results presented in Table XVI clearly show that there is a marked increase in the phenol content of the nodal and internodal tissues of all the 4 varieties, in response to infection by the fungus. It will, however, be noticed that the percentage of increase in phenol content in the nodal tissues of the red rot susceptible varieties is generally more than that of the moderately resistant ones. However, the total amount of phenol produced in the tissues of the susceptible varieties did not reach upto the level that the moderately resistant ones produced in response to infection.

It may be mentioned that the infection did not involve all the tissues of the inoculated nodes and internodes and hence it is possible that the actual increase in phenol content of the inoculated tissues might be more than what has been obtained in the present experiment.

TABLE XVI. Increase in phenol content of sugarcane stem, 7 days after inoculation with *Colletotrichum falcatum*

Cane variety	Cane portion used	Phenol content*		Increase in phenol content	Per cent increase
		Healthy	Infected		
Co. 445	Node	28.0	44.0	16.0	57.1
	Internode	16.5	27.0	10.5	63.7
Co. 331	Node	42.0	63.5	21.5	51.2
	Internode	18.5	30.5	12.0	64.8
Co. 1070	Node	60.0	85.5	25.5	42.5
	Internode	24.0	39.0	15.0	62.5
Co. 285	Node	68.0	98.0	30.0	44.1
	Internode	23.5	40.1	16.5	70.2

*In mg./100 gm. fresh weight.

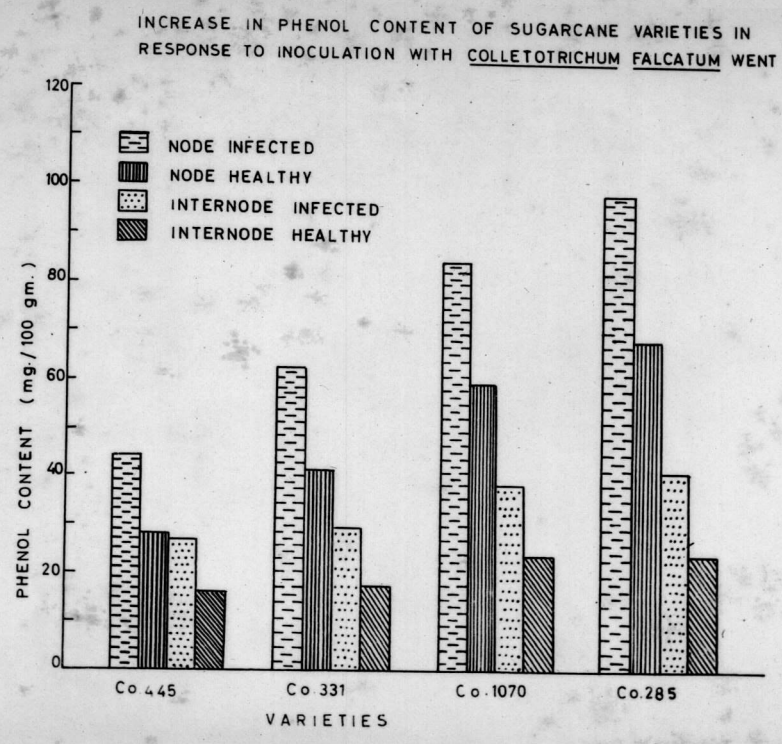

INCREASE IN PHENOL CONTENT OF SUGARCANE VARIETIES IN RESPONSE TO INOCULATION WITH COLLETOTRICHUM FALCATUM WENT
NODE INFECTED
NODE HEALTHY
INTERNODE INFECTED
INTERNODE HEALTHY
PHENOL CONTENT (mg./100 gm.)
120
100
80
60
40
20
0
Co 445
Co 331
Co 1070
Co 285
VARIETIES

Fig. 10

Relation of histochemical test for phenols to red rot resistance at the nodal region of sugarcane varieties:

A histochemical test using ferric chloride for detecting phenols has been employed by some investigators for the comparison of resistance to scab and wilt diseases in potato varieties (Johnson and Schaal, 1952 and McLean et al., 1961). As it has been proved in the previous experiments that the nodal region of sugarcane varieties moderately resistant to red rot infection through the nodal region contained more phenols than that in the susceptible ones, it was thought worthwhile to study whether the ferric chloride test for detecting phenols could be used for the comparison of red rot resistance in the nodal region of sugarcane varieties. The test was conducted as follows:

Cross sections (1.5 mm. in thickness) were cut from the 8th node (from top) of 4-5 canes selected at random from 4 cane varieties (Co. 445, Co. 331, Co. 1070 and Co. 285) in the month of August, by means of a Spencer Wood Microtome, Model No. 860. The sections were immediately placed in Petri-dishes containing 2 per cent aqueous ferric chloride solution. After 5 minutes the sections were removed and washed with three changes of tap water and studied for the development of green colour, which indicated the presence of phenols.

It was observed that the green colour developed near the outer edges of the sections and was more pronounced in the bud region. The sections of moderately resistant varieties showed a deeper green colour reaction than that of the susceptible ones and in most cases the green colour was observed throughout the surface of the sections of moderately resistant varieties (Fig. 11) thus suggesting that the ferric chloride test is indicative of varietal resistence to red rot infection through the nodal region of sugarcane and may be used to detect and eliminate the highly susceptible varieties.

Fig. 11. Transverse sections of the nodal region of sugarcane varieties after treating with ferric chloride for the detection of phenols.

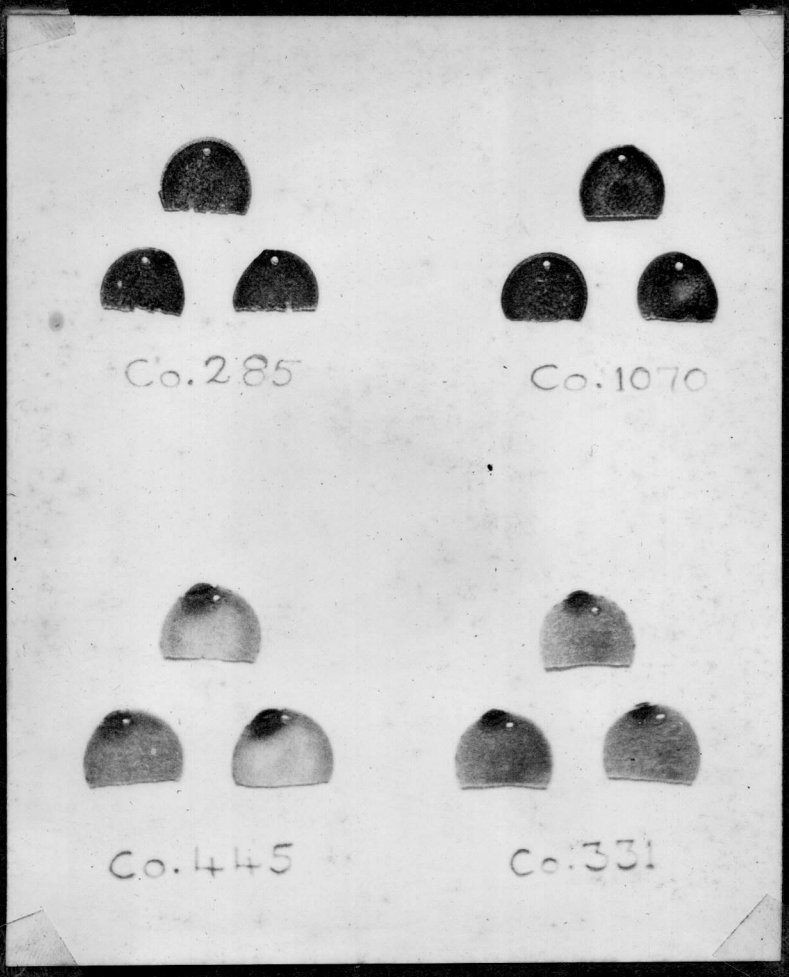


Fig. 11

V. DISCUSSION

The fact that Colletotrichum falcatum Went, the incitant of red rot of sugarcane, can infect through the nodal region of the growing sugarcane plant has been conclusively proved by the investigations of Butler and Hafiz Khan (1913), Chona (1950) and Steib and Chilton (1951).

Nodal infection experiments conducted during the present investigation showed that in most of the cases the infection originated at the leaf-scar region (the point of attachment of the leaf-sheath to the node) and in some cases through the root primordia. Only in very few cases the infection was found to have originated from the lateral buds. These observations are in close agreement with that made by Chona (1950). Steib and Chilton (1951) reported that the leaf-scar tissues, root-band and bud scales became infected as the leaf-sheath pulled away from cane stalk and that the fungus first developed on the leaf-sheath and finally infected the nodal tissues. By keeping apparently healthy cut canes at 70°C for 30 days, they observed that a high percentage of nodes developed red rot in the interior and that most of the initial points of infection were around the buds and leaf-scars. It may be mentioned here that, on splitting open the canes artificially inoculated at the nodal region during the present investigation, a number of cases of infection was found to have originated from the leaf-scar region and then spread to the other tissues of the node, including the lateral

bud. Such infections appeared to have originated from the eye-buds; but on careful examination of the infected node, the original point of infection could be invariably traced at the leaf-scar region.

Experiments to study the extent of natural infection of the nodes and leaf-sheaths of growing canes in the field, by C. falcatum showed that the fungus persisted in a latent form in the nodal regions of sugarcane and that the fungus could be isolated from the leaf-sheaths of all the varieties studied. Though these findings are in general concordance with those of Steib and Chilton (1951) and Sanchez-Navarrete (1962), the percentage of natural incipient infection of the nodes recorded during the present investigation, under the conditions prevalent at Delhi, was much less than what has been reported by the above investigators.

The leaf-sheath water of all the 6 varieties, used in the present study, markedly stimulated the spore germination of C. falcatum. This is ascribed to be due the presence of sucrose and laevulose, which were detected in the leaf-sheath water by chromatographic analysis. Lewton-Brain (1908) reported that sucrose was inverted by C. falcatum, but the actual consumption of sugar by the fungus was mainly as laevulose.

Experiments conducted to study the nature of resistance to red rot infection through the nodal region of sugarcane showed that structural and morphological characters like the

number of continuous vascular bundles from the stalk to the leaf-sheath and the number of silicated epidermal cells in the bud scales could not be correlated with resistance or susceptibility to nodal infection.

A number of investigators (Van Gundy and Walker, 1957; Gallegly and Niederhauser, 1958; Hadwiger and Hall, 1961 and Thomas and Orellana, 1962) have reported that the amino nitrogen content of the plant could be correlated with its susceptibility or resistance to infection by pathogenic microorganisms. Cornelison and Cooper (1940) observed that nitrogen in the sugarcane stem was stored or lodged in the tissues of the node immediately under the axillary bud, the root primordia and to a lesser extent in the protoplasm of the storage cells. Manocha and Chona (1963, unpublished) reported that increase in the total nitrogen content of the cane juice resulted in greater infection by the red rot pathogen. The results obtained during the present study showed that the nodes of sugarcane varieties susceptible to red rot infection through the nodal region contained more amino nitrogen than that of the moderately resistant ones, indicating thereby that the amino acid content might be a factor for determining the susceptibility or resistance of sugarcane varieties to nodal infection and spread of the pathogen. However, a large number of susceptible and resistant varieties will have to be analysed for their amino nitrogen content before any general conclusion is drawn in this matter.

Abbott (1938) made a comparative study of sugarcane varieties resistant and susceptible to red rot infection and concluded that the resistance principle was contained in the protoplasm and that the rate of spread of infection was governed by the characters of the cell contents rather than their walls. He further stated that preliminary determinations of phenolic compounds in the juices from the internodes of resistant and susceptible varieties showed that the former had higher total phenolic content than the latter. Parthasarathy and Vijayaserathy (1958) corroborated the above finding. The results obtained during the present investigation on the phenolic content of sugarcane varieties also showed that varieties moderately resistant to red rot infection had higher phenolic content in their nodes, internodes and leaf-sheaths than the corresponding tissues of the susceptible varieties. Histochemical test using ferric chloride also showed higher phenolic content in the nodes of moderately resistant varieties, as indicated by the intensity of green colour developed in the Transverse Sections of the nodal region tissues.

Phenolic compounds like chlorogenic acid, caffeic acid, catechol and protocatechuic acid have been reported to offer resistance in certain crop plants to parasitic diseases. In the present studies, chlorogenic acid has been detected in the nodal region of 4 sugarcane varities by paper chromatography. Though the quantitative differences in the chlorogenic acid content in the nodes of susceptible

and moderately resistant varieties was not studied by the chromatographic method, other tests conducted have indicated the presence of higher amounts of chlorogenic acid in the tissues of moderately resistant varieties.

Vorsatz (1942) modified the nitrous acid reaction for phenolics used by Hoepfner (1932) for the detection of chlorogenic acid in coffee bean extracts, and found it useful for differentiating a number of phenolics in solution. The Hoepfner-Vorsatz test gives a cherry-red colour reaction with chlorogenic acid and catechol as reported by Reeve (1951). In the present study, the extracts of sugarcane tissues also gave similar colour reaction with the above test: the extracts from moderately resistant varieties gave a more intense colour than the susceptible ones. Since catechol was not detected in the nodal extracts of sugarcane it is assumed that chlorogenic acid was mainly responsible for the cherry-red colour developed in the nodal extracts on applying the Hoepfner-Vorsatz test. Moreover, chlorogenic acid was believed to be chiefly responsible for the green colour reaction obtained with ferric chloride (Johnson and Schaal, 1952; McLean et al., 1956 and Lee and Le Tourneau, 1958). The intensity of green colour developed by applying ferric chloride solution to the nodal sections of moderately resistant varieties was also found to be more than that in the susceptible ones. It is, therefore, assumed that the major part of phenols in the sugarcane node is mainly in the form of chlorogenic acid

and that the moderately resistant varieties contained more chlorogenic acid than the susceptible ones.

Johnson and Schaal (1952) reported that the total amount of chlorogenic acid is not as important as the concentration in local areas. By applying the ferric chloride test, they observed that in some potato varieties resistant to scab disease, the chlorogenic acid was heavily concentrated in or near the lenticels which served as the natural avenues of entrance of the scab pathogen. The ferric chloride test conducted during the present studies also showed that the concentration of chlorogenic acid in the nodal region of sugarcane was more in tissues of the lateral bud and at the outer margins of the nodal sections. The concentration of chlorogenic acid, as indicated by the intensity of green colour in the ferric chloride test, was found to be more in the nodal region of moderately resistant varieties than that of the susceptible ones. The Hoepfner-Vorsatz test also gave similar results. This higher concentration of chlorogenic acid might account for their greater resistance to nodal infection than the susceptible varieties. Furthermore, the reason for obtaining only very few cases of infection originating from the lateral buds, as was observed in the nodal inoculation studies, is possibly due to the presence of high concentrations of chlorogenic acid in the bud tissues of both moderately resistant and susceptible varieties.

The nodal as well as internodal tissues of sugarcane varieties produced phenol in response to inoculation with Colletotrichum falcatum. The increase in phenol in the inoculated cut pieces was only very little, while in the standing canes the increase in phenol was very high. Though the moderately resistant as well as the susceptible varieties produced phenol in response to inoculation with the fungus, the total amount produced by the moderately resistant varieties was much more than that produced in the susceptible varieties. In all the 4 varieties studied, the nodal tissues produced more phenol than the internodal tissues. This increase in phenol at the infection site might be of special significance in helping the sugarcane plant to resist infection through the nodal region and also for localizing or checking the spread of the fungus in the cane tissues.

Chlorogenic acid in distilled water inhibited the spore germination of C. falcatum at concentrations above 250 ppm. with complete inhibition at 2000 ppm. When it was added to the sugarcane nodal leachate, there was only about 10 percent reduction in spore germination even at 1000 ppm. Further experiments on the effect of chlorogenic acid on the growth of the fungus could not be carried out due to the non-availability of pure chlorogenic acid.

Histochemical test using ferric chloride indicated that the nodal region of sugarcane varieties moderately resistant to red rot infection through the nodal region had

more phenol content than that of the susceptible varieties. Though the ferric chloride test may not be so sensitive as to differentiate cane varieties having slightly varying degrees of resistance or susceptibility to nodal infection, it is hoped that this test will be useful for the detection and elimination of cane varieties that are highly susceptible to red rot infection through the nodal region.

VI. SUMMARY

The reaction of 6 sugarcane varieties to red rot infection through the nodal region caused by Colletotrichum falcatum Went was studied. Cane varieties Co. 445, Co. 331, Co. 312 and Co. 1181 were found to be susceptible while Co.1070 and Co. 285 proved to be moderately resistant to nodal infection.

The fungus persisted in an incipient form in the nodal regions of apparently healthy stalks of sugarcane. A number of leaf-sheaths also yielded the fungus on isolation.

The leaf-sheath water (the water accumulated in the cavity between the leaf-sheath and stalk) collected from the above 6 varieties of sugarcane stimulated the spore germination of the fungus. The presence of sugars viz., sucrose and laevulose, detected in the leaf-sheath water by paper chromatographic analysis, is believed to be responsible for the stimulation of spore germination.

The study of the number of vascular bundles continuous from the stalk to the leaf-sheath and the number of silicated epidermal cells in the bud scales of sugarcane varieties did not reveal any correlation with their resistance or susceptibility to nodal infection.

There was no difference in the spore germination and growth of the fungus in the nodal extracts of the two moderately resistant and the two susceptible varieties studied.

The free amino acid content in the nodal tissues of the 2 red rot susceptible varieties, Co. 445 and Co. 331, was found to be more than that of the 2 moderately resistant varieties, Co. 1070 and Co. 285.

Chlorogenic acid, which has been reported to offer resistance in certain crop plants against infection by pathogenic microorganisms, was detected in the nodal tissues of the 4 sugarcane varieties studied by paper chromatography. Chlorogenic acid in distilled water inhibited the spore germination of C. falcatum.

The phenolic content (mainly chlorogenic acid) of the 2 sugarcane varieties moderately resistant to red rot infection through the nodal region was found to be more than that in the 2 susceptible varieties.

The nodal and internodal tissues of sugarcane produced phenol on inoculation with the red rot pathogen: the moderately resistant varieties producing more than the susceptible ones.

It is indicated that the low amino nitrogen content and high phenol content in the nodal tissues of cane varieties Co. 1070 and Co. 285 are responsible for their moderately resistant reaction to nodal infection by Colletotrichum falcatum. However, it is suggested that a large number of sugarcane varieties having varying degree of resistance or susceptibility must be tested in order to draw a general conclusion to establish such a correlation.

The histochemical test using ferric chloride for detecting phenols in the nodal region of sugarcane showed that

the phenol concentration was more in the lateral bud tissues and at the outer margins of the nodal tissue. The test also showed that the 2 cane varieties moderately resistant to nodal infection had more phenolic content in the nodal region than the 2 susceptible ones. It is, therefore, hoped that this test may be useful for the detection of cane varieties highly susceptible to red rot infection through the nodal region.

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