DISCUSSION

Papaya because of its importance as an article of food, vegetables and medicine and also its application in tanning and wool industries, has been investigated to a very considerable extent from different angles, viz., (a) agronomic aspects in relation to fruit raising, (b) storage, keeping quality and nutritive value, and (c) raising of disease-resistant of fruits. The researches of keeping papaya fruits in a disease free state have still not been investigated to an extent that makes clearer the understanding of factors that are primarily concerned not only in inducing pathogenesis in the fruit but also in the formation of resistance-promoting systems incited by the fruit itself. It is known that loss in fruits during storage is as high as 60%–80% due principally to Fusarium solani and Colletotrichum gloeosporioides in Noya-banpla and Haflong variety and other fungi inspite of the environmental atmosphere being controlled.

It will be seen from the results that papaya varieties, viz., Noya-banpla and Haflong, slightly differ in their susceptibility to infection by F. solani and C. gloeosporioides. It is also to be noted that soil microflora of the papaya plantation of both the varieties abounding in disease inducing fungi, bacteria and actinomycetes contribute towards inoculum potential of the fungi specifically pathogenic to the fruits. Risbeth (1955) studying the Fusarium wilt of banana in Jamaica found that the infected banana plant is direct source of infection spreading to other plants and that infected soil plays a part in dispersal. The assessment of the resistance of
two varieties of papaya fruit by inoculation experiments showed that Haflong variety of papaya was more susceptible than Noya bangla variety. Inoculation experiments of papaya fruits of Haflong and Noya-bangla varieties and storage at different temperatures also demonstrate that maximum infection takes place at 25°C on 15th day of inoculation but the temperatures below and above this range, there is decrease in the extent of infection in both the varieties infected by *F. solani* and *C. gloeosporioides*. Inoculation experiments further demonstrated that in relation to relative humidity (R.H.) maximum development of papaya fruit rot by the two pathogens - *F. solani* and *C. gloeosporioides* was noted at 100% R.H. The rate of disease development was found to be proportional to the humidity level. Thus, with decline in R.H. there was a parallel decrease in disease development. Maximum rot development by both the pathogens took place on 12-15 days.

Inoculation trials indicated that injury to the fruits was necessary for successful colonization of the pathogens. Similar results regarding pathogenesis of various fruits have been reported in literature (Chand et al., 1968; Wood, 1967; Tandon and Mishra, 1969); and that indicates the importance of avoiding injury to fruits of papaya in storage and transit.

Srivastava and Walker (1959) have reported similar results with *Rhizopus stolonifer* in case of sweet potatoes. The degree of ripeness of papayas influenced the disease development by *F. solani* and *C. gloeosporioides*. Ripe fruits were most susceptible to infection by the pathogens than the semi-ripe fruits, and green fruits were more resistant.
It is thus evident that once infection is established by direct inoculation at wounds in the papaya fruits, the growth of the pathogens inside the tissues of the host and disease reactions induced develop in consequence.

The growth of the pathogens inside the tissues of the host is influenced by the nutritive composition of the fruit itself as the fruit (according to Hlth. Bull., 1941, No. 23, 39) is stated to contain carbohydrates, protein, minerals, vitamin, calcium, phosphorous and iron (page 3). Studies in vitro on the growth rate of *Fusarium solani* and *Colletotrichum gloeosporioides* indicate that the growth is influenced by the concentrations and limits of the different nutritive factors in a way that may parallel the growth of the fungi in vivo. Gregory and Horne (1923) have indicated that the growth rate of the apple-attacking fungi in vitro parallels the growth of these fungi in vivo, growth rate in apples being measured in terms of radical advance of the fungi inside the apple tissues. This hypothesis has limited application, because the biological systems differ quite considerably from what appears in vitro (Baishya, 1965).

The experimental results with *F. solani* and *C. gloeosporioides*, however, indicate that the growth rate is influenced by the carbohydrates, nitrogen, salts, trace elements, auxins, vitamin and pH. The fact that the papaya extracts from the core and cortex of papaya fruits when added to a synthetic nutrient medium enhanced the growth rate of the fungi to a marked extent and not the papaya extract from the periderm which, on the other hand, is inhibitory to the growth of the fungi.
point to the possibility of significant correlation between the host substrate and the pathogens. It has not, however, been possible to correlate the growth of the fungi inside the tissues of the host with the nutritive composition of the fruit itself as there has been no appropriate method unlike that in apples, characterised by soft rot measuring the rate of advance of the hyphae into the tissues. The nature of this aspect of rot in papaya fruits caused by F. solani and C. gloeosporioides is affirmed by the evidence that these fungi produce pectinolytic enzymes not potent enough to macerate tissues quickly or even caused rapid changes in viscosity of a standard pectin solution during hydrolysis. Papaya fruits are rich sources of pectins (Krishnamurti and Giri, 1949), the factor which may be responsible for not promoting the formation of active pectinolytic enzymes (Baruah, 1941). Indications are, however, given of slight cellulolytic action of F. solani and C. gloeosporioides extracts. Further experiments on enzymatic activities on F. solani and C. gloeosporioides have also shown that F. solani normally does not exhibit oxidase or peroxidase reaction, whereas the fungus grown in a medium containing a phenolic substance (Bavandamm, 1928 - reaction) produces an enzyme capable of oxidising the phenol, but there were absence of any oxidase activity with the fungus C. gloeosporioides grown in a medium containing a phenolic substance. Phenolic substances are known to play an important role in the suppression of various fungal diseases of crop plants (Kuc et al., 1956; Clause, 1961). In case of Alternaria solani, Bhatia et al. (1972) have reported a higher concentration of phenolics in resistant variety of
tomato plants than in susceptible ones. The utilization of this compound after 12 days of infection may be due to assimilation or degradation of this compound during pathogenesis. By studying the phenolic substrates of potatoes Baruah and Swain (1959) also isolated and identified, besides tyrosine identified by Isherwood (1937), a number of phenolic compounds such as chlorogenic acid, caffeic acid, scopoletin, ascleutin and quercetin. It is thus probable that a complex mechanism operates itself during pathogenesis in the fruits as evident during the histological changes during infection by the formation of dark granular substances in the cell adjoining the zone of infection. This has been further strengthened by ultra-violet analysis of infected fruits showing definitive zones of fluorescence increasing in intensity and specific colouration as infection progresses. Hughes and Swain (1960), also state that the potatoes infected by Phytophthora showed a large increase in the amount of scopoletin in the tubers and suggest that this substance may prove highly toxic to the pathogens. Experimental findings indicate that F. solani and C. gloeosporioides not only secrete enzymes of the nature already indicated above but also toxins which may have a significant contributory influence on the disease development and development of fruit rot by the two pathogens. This result is in agreement with Ludwig's findings (1957).

The principal features associated with Fusarium and Colletotrichum infections of papaya fruits are most marked, in the case of changes in the carbohydrate content, nitrogen content, ascorbic acid, iron, calcium and phenols. These
substances constitute the preponderant nutritive factors in the healthy tissues as well.

Suzuki (1957) studying the Murasaki-mompa disease caused by Helicobasidium mompa in sweet potatoes distinguished four types of infections and also defence reactions against fungal attack. He reported "at the first stage of infection, the middle lamellae of cork layer cells are penetrated by the fungus, as has been shown before. Pectic material in the middle lamellae swells when the hyphae come in contact with it and the pH value is decreased. After passing through the cork layers the fungus comes into contact with the starchy parenchyma. In susceptible varieties these tissues are then macerated by the action of fungal enzymes. Itaconic acid is isolated from such tissues (Araki et al., 1957). The decrease of pH in these tissues is mainly due to the accumulation of chlorogenic acid and caffeic acid and to Itaconic acid produced by the fungus. When treated with ruthenium red in the early stage the pectic substance of the invaded tissues in contact with the hyphae - is stained yellow, and the tissues beneath the phellodgen are stained carmine red. Ferric chloride - potassium ferricyanide solution stains pectic materials in the cork layers blue. No colour reaction occurs in pectic materials produced post-infectionally."

The appreciable decrease in the total sugars in the papaya fruits during infections by both the pathogens (F. solani and C. gloeosporioides) may be due to (a) the direct utilization by the fungus concerned for its growth, (b) an increase rate of
respiration of the host tissues during infection causing the oxidation of larger quantities of carbohydrates (Horsfall and Dimond, 1960; Cochrane, 1958). Utilization of glucose and maltose during pathogenesis has also been noted by Kapoor and Tandon (1969, 1970) on tomato fruits infected by Drechslera australiense. Selective loss of certain sugars probably occurs due to their utilization by the pathogen for its growth and other metabolic activities. In the same way nitrogen content of the papaya fruits during infection by the pathogens showed marked decrease. This is attributable directly to fungal metabolism. The ascorbic acid content in papaya fruits decreases during storage but the decrease is more rapid in the infected fruits. It has been shown in the healthy potatoes (Smith and Gillies, 1940), the ascorbic acid decreases during storage to half its value in a month, and DHA increases though not in proportion to AA loss. It is suggested that this may be due to the conversion of DHA to some other oxidised form which is irreversible. But the increased rate of disappearance during infections may be due to the break down of tissue of the host and the increase in the oxygen content of the tissues causing autoxidation of the ascorbic acid. The high iron value in the papaya fruits is also a possible source of an oxidising agent of ascorbic acid (Kellie and Zilva, 1935) which may account for rapid decrease in the ascorbic acid content in the papaya fruits during infections. In assessing the metal ion content of papaya fruits it becomes evident that the disappearance of ascorbic acid during infection and the parallel decrease in the iron content, that the tissue iron while catalysing the oxidation of .
ascorbic acid may also be catalysed in its turn by ascorbic acid in forming metal-protein complexes in the papaya fruits thereby reducing the free iron and ascorbic acid values during infection. The iron-protein chelated compounds are produced during infection of plant tissues causing toxicity and resistance in plants (Gaumann and Kern, 1953). This is also possible in the case of infected papaya fruits or the iron molecules may be utilised in the formation of enzyme proteins by the fungus concerned or the host tissues with iron as prosthetic group.

The exact mechanism by which the biochemical reaction takes place in browning of tissues, which is a characteristic features of hypersensitivity in the host, is not clearly known. Horsfall and Dimond (1960) after histological observations of necrosis (Browning and Drying) in the injured tissues of infected plants, drew the general assumption that - "(i) polyphenolic compounds are oxidised by polyphenol oxidase which may exist in a latent state in the intact plant tissue and be activated on exposure to pathogenic infection; (ii) the oxidised polyphenols, now quinones, are condensed to form polyquinoid structures or sometimes react with amino-acids or proteins to form melanin like substances. The net effect of these reactions may constitute defence mechanism of the host by forming a barrier." Infected potato tissues of Phytophthora infestans show a considerable increase in the activity of polyphenol oxidase and the amount of polyphenols (Rubin et al., 1947; Rubin and Axenova, 1957). Gaumann et al. (1953) isolated a browning factor from culture filtrates and called it 'Vasinfuscarin' and suggested that it was enzymatic in character.
In the present investigation, significantly enough, tests carried out on papaya fruits using phenols reagents and ultraviolet fluorescence showed difference in the phenolic concentration in the various zones of papaya fruits. There was also a definite increase in concentration of certain fluorescent compounds adjacent to the site of infections. The results of inoculation tests on the two varieties of papaya fruits and the phenolic composition confirmed the previous findings that there is a positive relationship between susceptibility to pathogenic infection by *Fusarium* and *Colletotrichum* and a low phenol content. Generally the concentration of phenol is high in the periderm below epidermal layers but normally low in the mesocarp cortex; hence the pathogen concerned, once it has forced an entry through periderm lesions or other wounds is able to spread without much resistance from the soft tissues which are partially destroyed by the pectinolytic enzymes of the fungal mycelium. The decrease in the sugar concentration during infection by *Fusarium* and *Colletotrichum* may also contribute to a lowering of osmotic pressure of the cells and subsequent case of breakdown of cell walls.

Although no hard and fast rule can be applied to the selection of resistant varieties or the control of wastage in storage due to *Fusarium* and *Colletotrichum* infection of papaya fruits, the correlation between percentage of skin lesions, phenolic concentrations and the degree of resistance to attack is significant from a practical point of view. The general picture of the vast changes even in the few host substrates studied in this work shows that the variations in the host
parasite relationship and disease reactions are brought about by the changes in a small group of chemical systems in the host and the parasite and not due to an overall change. The striking phenomenon of the increasing concentration of certain phenolic compounds in the host tissues and the production of a strong phenol oxidase system by the pathogens is also a clear indication of the mechanisms of attack and defence in diseased systems.

It would be of interest to carry out further researches into the qualitative and quantitative changes in the papain and also in the primary metabolic compounds such as amino-acids, carbohydrates, organic acids, and secondary components such as phenols and alkaloids and a graphic representation of these quantitative changes during incubation of a parasite should help in the better understanding of resistance or susceptibility of a crop to a specific disease.