On Sorghum Rio this isolate produced mild mosaic symptoms in the entire leafblade (figure 3). None of the 250 lines of sorghi tested produced local lesions with the new isolate. There is no report of local lesion host with SCMV strain H until today.

Yellowing and necrosis was observed on primary leaves of five lines of green gram one week after inoculation. Though the necrosis resembled the translocated necrotic local lesions[17], the virus could not be recovered. The reaction of the rest of the varieties is shown in table 1.

Physical properties: The dilution end point was between 10^3 and 10^4, the thermal inactivation point between 56 and 58 C and longevity *in vitro* for 72 hr at room temperature (32°C); and these were in accordance with the other SCMV strains.

In three experiments, both *R. maidis* and *M. Sacchari* could transmit the disease from Rio to Rio seedlings showing 50% and 65% transmission, respectively.

No seed transmission was observed in any of the 1200 sorghum seedlings even four weeks after germination.

The symptomatology on sugarcane and sorghum differentials, aphid transmission and physical properties of the present isolate resemble very closely those of strain H reported from other countries[3,7,18].

In the present study, SCMV strain H is thus reported for the first time from India.

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POST-INFECTION CHANGES OF CHITIN IN BANANA FRUITS

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THE convential method of determining fungal infection in plant tissues is by transferring a portion of host tissues to a suitable medium and allowing the growth of the organism. However, determination of fungal presence through measurements of fungal chitin (cell-wall constituent of most fungi) may have great promise. The assay procedure, which is more rapid than the plating method, is also considered to be more sensitive and needs fewer samples for analysis. This method is based on the alkaline depolymerisation and deacetylation of chitin (a linear polymer of β 1,4 linked N-acetyl-glucosamine) to glucosamine which on deamination yields aldehydes that are colorimetrically estimated. Although glucosamine may be present in many plant tissues, it does not interfere with the determination of the total glucosamine obtained from chitin, since the amount present is known to be characteristics of the species.

Golubchuk *et al*[2] studied the chitin content of hydrolysed crude fibre preparation of 5 varieties of wheat with varying degrees of fungus invasion and concluded that the measurement of chitin could offer promise in the evaluation of deterioration of wheat on storage, but the analytical procedure needed improve-

ment. Arima and Uozumi[3] successfully used chitin analysis as a measure of mycelial weight in Koji. The object of the present experiment is to explore and adapt the method to measure the extent of fungal growth in fruits on storage.

With this in mind the changes in the chitin content in banana fruits ('Champa' and 'Kanthali' variety) artificially inoculated by *Botryodiplodia theobromae* Pat. and incubated for different periods of time were studied.

The method of chitin estimation by alkaline hydrolysis[4] was followed with certain modifications. Pulp tissues of banana were first dried at 104°C for 24 hr and then ground to powder (40 mesh) in a stein mill and bottled until used. Pulp powder (100 mg) was transferred into glass centrifuge tubes (15 ml) and mixed thoroughly with 5 ml of acetone and centrifuged at 8000 rpm for 10 min. The residue was washed with distilled water, treated with 3 ml of concentrated KOH (120 g in 100 ml water) and the mixture kept at 130°C for 1 hr with frequent agitation for complete hydrolysis. It was then cooled and mixed with 3 ml ice-cold 70% ethanol to precipitate the chitosan formed. The mixture was covered with 1 ml celite suspension (1 g celite 545, in 20 ml 70% ethanol at ice temperature kept for 15 min). It was then centrifuged (8000 rpm) and the residue washed, first with 5 ml ice-cold 40% ethanol and then three times with distilled water. The final residue was suspended in 1.5 ml. distilled water and kept for assay.

The assay method of chitosan was based on its deamination to glucosamine and expressed as μg glucosamine. As such, a set of external standard solution of glucosamine-HCl in distilled water was prepared to get concentrations of 100, 75, 50, 20 and 10 μg/ml. These solutions and the water blanks were incorporated into the assay. The chitosan suspensions as well as the external standard solutions (1.5 ml) were mixed with equal volume of 5% $NaNO_2$ and 5% $KHSO_4$ and kept for 15 min with periodic shaking. The mixture was finally centrifuged. Two samples (1.5 ml each) of the supernatant were taken in separate tubes and mixed with 0.5 ml $NH_4SO_3NH_2$ (12.5%) for 5 min. Half a ml of MBTH (0.5 aqueous solution of 3-methyl-2-benzothiazolone hydrazone hydrochloride) was then added and the mixture was heated in boiling water for 5 min. It was then cooled in ice and mixed with 0.5 ml $FeCl_3$ (0.5%) and allowed to stand for 30 min. The optical densities of the blue solutions formed were measured at 650 m/μ.

The amount of glucosamine obtained from chitin of infected fruits were quantitatively estimated from the standard curve of the graded external glucosamine-HCl solutions. From table 1 it is evident that following the infection by *Botryodiplodia theobromae*, chitin content increased considerably with the storage time in both the cultivars, showing their maxima after 6 days of incubation. Among the two cultivars, 'Champa' showed significantly higher chitin content than that in 'Kanthali'. This may be due to higher hyphal growth occurring in 'Champa' when inoculated with *B. theobromae*. In control fruits the amount of chitin content was almost negligible.

Table 1 *Chitin contents of healthy (H) and infected (I) pulp tissues of 'Champa' and 'Kanthali' cultivars of banana fruits caused by B. theobromae Pat. after 2, 4 and 6 days of incubation*

No. of days	Champa	Kanthali
2 (H)	6.0	4.5
4 (H)	6.2	4.7
6 (H)	6.5	4.9
2 (I)	11.5	10.4
4 (I)	18.4	15.2
6 (I)	41.0	38.5

'H'—Healthy; 'I'—Infected,
Mean value of chitin content (μg/100 mg of dry pulp)
Champa: Healthy – 6.23 *Kanthali*: Healthy – 4.70
Infected – 23.63 Infected – 21.37
S.Em = $\pm$0.30/μg; C.D. (1%) = $\pm$1.11 μg
Days of incubations: 2 – 8.10; 4 – 11 13; 6 – 22.73 S.Em = $\pm$0 28 μg. C.D. (1%) = +0.82/μg C.V = 4.27%.

Chitin content was reported by a number of investigators (4–6) in fungus infected tissues of grains and leaves. Such increase was mostly directly proportional to the fungal growth within the tissue of the host depending on the rate of growth of individual species under the available condition.

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ROLE OF CORPORA-ALLATA AND BRAIN ON THE CARBOHYDRATE LEVEL OF HAEMOLYMPH IN *LOHITA GRANDIS* GRAY (PYRRHOCORIDAE: HETEROPTERA: HEMIPTERA)

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THE insect haemolymph, in addition to transporting nutrients and waste products between tissues can have a significant storage function, substances getting accumulated or utilised depending on the circumstances. But very little information is available on the distribution of solutes between the plasma and the tissues, although it is clear that the distribution can be very uneven and can change quickly. Such changes are of prime importance in regulating the process of metabolism. Further, the hormones from different sources (*e.g.* brain, corpora-allata, corpora-cardiaca) are also transported by the haemolymph and these play a major role in its biochemistry[1]. L'Helias[2] first pointed out that in *Carausius* (a wingless stick insect), allatectomy brought about a steep decline in the level of blood sugar. According to Ralph and McCarthy[3], the injection of saline extract of brain caused a rapid increase in the level of trehalose of haemolymph in *Periplaneta americana*. Goldsworthy[4], in *Locusta*, noted that removal of ceRebral neurosecretory cells resulted in a marked accumulation of carbohydrates in the haemolymph and commented that it was due to the metabolic changes brought about in the absence of cerebral neurosecretion. Gade[5] observed species specificity, to the role of neurosecretion and hormones of corpus allatum/corpus cardiacum, on the metabolism of carbohydrates and observed that the neurosecretory corpora cardiaca and corpora allata complex of *Carausius* contained both adipokinetic and hyperglycemic factors that were responsible for the increased levels of lipids and carbohydrates in *Locusta* and *Periplaneta* respectively. The present investigation was undertaken to determine the level of carbohydrate in the haemolymph of *Lohita grandis* after removal of brain and corpora-allata from the insect. Effect of application of Juvenoid (JHa) on carbohydrate level in allatectomized insects was also studied.

Adults were collected from the colonies reared in the laboratory by the method of Mandal *et al*[6]. The corpora-allata was removed by the methods of Stay and Tobe[7] and brain-cauterization performed by the technique of Girardie[8]. In all cases during operation, the insect's Ringer solution used was mixed with a few crystals of phenyl thiourea and the operated area was immediately sealed with the sterile "Bees-wax" impregnated with phenyl-thiourea. The juvenile hormone analogue (JHa) used was N-(2,5-dichlorophenyl)-3,7-dimethyl-2,6-octadienylamine obtained from Prof. A. M. De Oliveiro Filho (Brazil) and was injected into the allatectomized insects at a dose of 20 μg in 10 μl acetone by a tuberculin microsyringe (Hamilton, USA). The control insects received only 10 μl of acetone/insect. For each treatment the experimental insects were sacrificed at 24, 48 and 72 hr after treatment. The haemolymph was collected by the graduated glass capillary tube and stored at 0°C for biochemical analysis. The total carbohydrate level of haemolymph was estimated according to Mordue and Goldsworthy[9] while the trehalose was estimated according to Roe[10]. Glucose was estimated by the glucose oxidase method[11]. The volume of haemolymph was determined by the dye dilution technique[12]. The contents of carbohydrates were expressed per mg haemolymph protein; and the total protein content was determined by the methods of Lowry *et al*[13]. The experimental data were analysed statistically by the students-*t*-test and Duncan's multiple range test.

It was found that allatectomy resulted in a sharp decline of the total carbohydrate, trehalose, and glucose level in the haemolymph as compared with the sham-operated control cases (table 1). The post-treatment exposure also exerted a significant effect *i.e.* the decline was more pronounced in the 72 hr than either in 24 hr or in 48 hr after operation (table 1). The volume of haemolymph increased significantly ($P < 0.01$) after the removal of corpora allata (table 2). Application of JHa into the allatectomized insects reversed the effects of allatectomy *i.e.* it increased total carbohydrate, trehalose and glucose levels and a drop in the volume of haemolymph compared with the allatectomized insects (tables 1 and 2) was noticed. Compared with other treatments, the removal of brain resulted in a significant increase ($P < 0.01$) in the total carbohydrate and glucose contents and fall in tre-