Conidia from mildewed leaves were dusted on to healthy plants. Sporulating colonies developed on such plants after 7 days of inoculation.

Conidia germinated well and produced a single germtube on glass slide at 100% relative humidity when incubated for 24 h in the dark. The pathogen was identified as *Oidium* sp. (conidia 10–20 × 20–60 $\mu$m in long chain). This is the first report of *Oidium* sp. on *M. cochinchinensis* from India[1–3].

The authors are grateful to Dr B. C. Sutton, CMI, Kew, England for identification of the fungus. The infected leaves have been deposited at CMI (Herbarium IMI No. 314780).

13 July 1987; Revised 2 November 1987

1. Bilgrami, K. S., Jamaluddin and Rizwi, M. A., *Fungi of India, Part I*, Today and Tomorrow's Publishers, New Delhi, 1979, pp. 457.
2. Bilgrami, K. S., Jamaluddin and Rizvi, M. A. *Fungi of India, Part II*, Today and Tomorrow's Publishers, New Delhi, 1981, pp. 140.
3. Mukerji, K. G. and Bhasin, J., *Plant diseases of India: A source book*, Tata McGraw Hill, New Delhi, 1986, pp. 468.

## SEX DETERMINATION IN THE LARVAE AND PUPAE OF SOYBEAN LEAF MINER, *APROAEREMA MODICELLA* DEVENTER

O. P. SINGH and K. K. NEMA
*Jawaharlal Nehru Agricultural University, College of Agriculture, Sehore 466 001, India.*

SOYBEAN leaf miner, *Aproaerema modicella* Deventer (*Stomopteryx subsecivella* Zeller) is a serious pest of soybean and groundnut in Madhya Pradesh, Maharashtra, Gujarat, Tamil Nadu and Andhra Pradesh[1–4]. Gujrati *et al*[5] studied the biology of the pest and Mohammad[6] reviewed the available literature on the pest, but studies on sex differences in larval and pupal stages are lacking. Determination of sex in adults by examining the genitalia is impracticable as many of the adults are injured and become unfit for experiments. Attempts were, therefore, made to determine the sex at larval and pupal stages on the basis of easily distinguishable characters.

A laboratory culture of the pest was maintained during the rainy season (July to October) of 1986 at Sehore in Madhya Pradesh. Larvae having a pair of spots on the 5th abdominal segments and without spots were kept in separate petri dishes (10 cm dia). Pupae developing from these larvae were further examined for their genital openings, and finally adults formed from these pupae were dissected for confirmation of male and female sexes.

While rearing, two types of larvae were observed: one having a pair of spots on the 5th abdominal segments and in others these spots were absent. These spots appeared in the second instar as pinkish-brown patches, which changed to dark pinkish-brown in the third instar and brownish-violet in the fourth. The larvae having spots finally developed into male adults, while larvae without spots emerged as female adults. While studying the biology of *A. modicella* on soybean, Gujrati *et al*[5] found two types of larvae, one with spots on the 5th abdominal segments and in others these were absent, but they could not identify sexes on the basis of these spots. However, Mohammad[6] reported that the male larvae have pinkish gonads underneath the dorsum of the 5th abdominal segments, but he did not mention the varying colours of gonads in different instars. After pupation, pupae were observed for genital openings. It was found that in female pupa a slit-like genital opening was situated on the 8th sternum and anal opening was located on the 10th sternum. The 8th and 9th segments were not completely demarcated and the intersegmental line was also rudimentary between the 9th and 10th sterna. Thus the pupa had a composite sterna of 8th, 9th and 10th segments. In the male pupa, the genital opening was present in the 9th sternum of the abdomen. The intersegmental line between the 8th and 9th segments was prominent, whereas it was not prominent between the 9th and 10th abdominal segments. Male pupae also had two small pad-like structures on either side of the anal slit on the 9th abdominal sterna, but in female pupae, these pads were absent. The female pupae measured 6.15 mm in length, while the male pupae were 5.21 mm long. Arrangement of genital openings in *A. modicella* was found to be similar to that of *Spilosoma obliqua* Walker and *Spodoptera litura* Fabricius[7,8].

The authors thank Dr D P Nema for encouragement and ICAR, New Delhi for financial assistance.

13 July 1987; Revised 29 September 1987

1. Khan, M. I. and Raodeo, A.K., *J. Maharashtra Agril. Univ.*, 1979, **4**, 116.
2. Krishnamurthy Rao, S., Rangacharulu, P. and Yesudar, T., *Andhra Agric. J.*, 1962, **3**, 202.
3. Singh, K. J. and Singh, O. P., *Indian J. Plant*

*Protect.*, 1983, **11**, 107.
4. Urs, K. C. D. and Kothari, K., *Madras Agric. J.*, 1976, **63**, 371.
5. Gujrati, J. P., Kapoor, K. N. and Gangrade, G. A., *Entomologist*, 1973, **106**, 187.
6. Mohammad, A., Occasional paper No. 3, Groundnut Improvement Programme, ICRISAT, Patancheru, India, 1981, p. 33.
7. Rathore, Y. S. and Verma, J. K., *Pantnagar J. Res.*, 1977, **2**, 233.
8. Singh, O. P. and Ali, S. I., *Agric. Sci. Digest*, 1988, (in press).

## POLYPLOIDY, NUCLEAR DNA AND HECOGENIN IN FOUR SPECIES OF *AGAVE*

SUCHITRA BANERJEE and A. K. SHARMA
*Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700 019, India.*

STEROIDAL compounds including sitosterol, diosgenin, hecogenin and tigogenin have been analysed in different species of *Agave*[1–14]. The compounds occur in different parts of the plants especially the leaves. Several species of *Agave* have recently been subjected to detailed cytological analysis in addition to the quantitation of the amount of DNA[15]. There are ample records in literature of variations in chemical content of the plant concomitant with polyploidy[16–19]. In view of the existence of diploid to polyploid species in this genus and the steroidal sapogenin so far recorded, the present study was undertaken.

Four species viz., *Agave angustifolia* Haw. var. *marginata* Hort., *A. americana* L. var. *marginata* Trelease, *A. sisalana* Perr. and *A. decipiens* were selected. For chromosome study, paradichlorobenzene pretreatment of root tips for usual schedule of orcein technique[20] was applied.

For estimation of *in situ* 4C nuclear DNA amounts, acetic acid: ethanol fixative and Feulgen staining technique was followed. Cytophotometric estimation of DNA from metaphase was carried out in a Reichert Zetopan microspectrophotometer following single wavelength (550 mm) method[20].

Sapogenins from all the four species were extracted from leaves following the usual procedure of drying, powdering acid hydrolysis followed by extraction with petroleum ether, the residue being dissolved in chloroform. For qualitative separation of sapogenin TLC techniques[21] were adopted comparing with standard sample under UV light and the purity of the hecogenin extract was confirmed through IR spectrophotometry.

For quantitative estimation, standard curves were made by both direct and preparative methods[22,23]. By comparing the two curves, the loss due to preparatory method could be calculated. Known amounts of the crude extract were applied to one spot on each plate for identification of hecogenin spot of the crude extract. The normal procedure for spectrophotometric quantitation was adopted at 390 nm. The concentration of hecogenin was worked out for each sample from the standard curve by extrapolation of values using the formula: $a = (c/d) \times b$, where $a$ is the amount of hecogenin present in total crude sapogenin extract, $b$ the total amount of the crude extract, $c$ the concentration of hecogenin obtained from the standard curve and $d$ the amount of crude extract applied in each plate. The percentage of hecogenin was calculated following the formula $(a/e)$ 100 where $e$ is the initial dry weight of the sample.

The four species revealed four different numbers viz. 60, 120, 150 and 180 chromosomes in the somatic cells indicating different degrees of polyploidy (table 1).

The amount of nuclear DNA differed to a certain extent among the four different species, the least amount i.e. 0.0993 units being recorded in the diploid species — *A. angustifolia* Haw. var. *marginata* Hort. ($2n = 60$) and the highest amount i.e. 0.1945 units in the hexaploid species — *A. decipiens* ($2n = 180$). Between the tetraploid and pentaploid species, the difference in the amount of DNA was rather negligible (table 1, figure 1), despite heavy difference in chromosome number and length between these two species. The absence of any direct multiplication of DNA amount in consonance with the multiplication of the chromosome number is an index of the differential DNA content in different species at the diploid level[24–26]. The structural alterations of chromosomes have also been recorded[27].

The presence of hecogenin was recorded at $R_f$ 0.5/0.52 in the diploid, tetraploid and pentaploid species. In the hexaploid, the presence of hecogenin in trace amounts was noted.

Besides, tigogenin has been observed at $R_f$ 0.74/0.76 in the diploid, tetraploid and pentaploid species, but the colour intensity of the spots was different in the different species. In the hexaploid species, *A. decipiens*, this spot was absent. E-sitosterol has been noted at $R_f$ 0.8/0.83 in all the four