

**Table 2** Percent of male moths with different number of spines on aedeagal cornuti, obtained from the light trap during crop seasons, ICRISAT Center, 1976-77.

Spines on aedeagal cornuti No.	No. of moths (%)		
	Jul.-Oct. <sup>a</sup> (n = 65)	Nov.-Jun. <sup>b</sup> (n = 98)	Mar.-Jun. <sup>c</sup> (n = 79)
10	7.7	9.2	5.1
11	23.1	31.6	34.2
12	46.2	32.7	32.9
13	20.0	23.4	21.5
14	1.5	3.1	6.3
15	1.5	0.0	0.0

n: No. of male moths obtained and studied

a: Crops available – groundnut, sorghum, and pearl millet

b: Crops available – pigeonpea, and chickpea

c: Crops available – rabi groundnut, sorghum, and weeds

vary more or less in the same narrow range with the host plants; and therefore, it should not be taken as criterion to name subspecies of *H. armigera* feeding on different crops, as has been done by Bhattacharjee<sup>1</sup>.

We, further, got identified the species of *Heliothis* in Andhra Pradesh, India by sending the moths obtained from different hosts to Dr D. F. Hardwick, Entomological Research Institute, Canada. There were three *Heliothis* species in the lot namely-*Heliothis* (= *Helicoverpa*) *armigera* on almost all host plants, *H. peltigera* mainly on safflower and the weed *Acanthospermum hispidum*, and *H. assulta* on the weed *Datura metel*. It needs to be mentioned here that the generic name *Helicoverpa* coined by Hardwick<sup>4</sup> for *Heliothis* has not been approved by the International Commission on Zoological Nomenclature, and there is a representation by Nye<sup>5</sup> following a referendum among the participants of the "International Workshop on *Heliothis* Management" held in November 1981, at ICRISAT, Patancheru, A.P., India, that the well established *Heliothis* spp names including *armigera*, *peltigera*, and *punctigera* should continue to be used.

The authors are grateful to Dr D. F. Hardwick, Entomology Research Institute, Ottawa, Canada, for identifying the species.

29 June 1984

1. Bhattacharjee, N. S., *Entomol. Newslett.* New Delhi, 1972, 2, 3.

2. Bhattacharjee, N. S. and Gupta, S. L., *J. Nat. Hist.*, 1972, 6, 147.

3. Vaishampayan, S. M., *Curr. Sci.*, 1976, 46, 27.

4. Hardwick, D. F., *Mem. Ent. Soc. Canada.*, 1965, 40, 247.

5. Nye, I. W. B., *Proc. Int. Workshop on Heliothis Management.*, 1982, pp. 3. ICRISAT.

## EFFECT OF OX BILE ON THE FORMATION OF CALCAREOUS CORPUSCLES DURING *IN VITRO* GROWTH OF *HYMENOLEPIS MICROSTOMA*

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THE scoleces of three species of cestodes *e.g.* *Hymenolepis microstoma*, *H. diminuta* and *H. nana* remain attached to the bile duct, duodenum and posterior third of the gut<sup>1</sup>, respectively. In the former species, the major portion of the mature and all the gravid proglottids, however, remain hanging in the duodenum. The different regions of the gut in which these cestodes inhabit contain a decreasing gradient of bile and pancreatic juice. It has been demonstrated *in vitro* by us that pancreatin apparently has no effect on the numbers and sizes of the calcareous corpuscles<sup>2</sup>. In the young adults of *H. microstoma* these structures increase in numbers as soon as they come close to the bile duct<sup>3</sup>. The concentration of bile which produces maximum increase in the formation of calcareous corpuscle in cestodes, remained a gap in our knowledge. In the present *in vitro* experiment, the effects of various concentrations of ox bile (rich in bile acids and bile salts) on the formation of calcareous corpuscles in *H. microstoma* was studied.

The Eagle's basal medium containing horse serum and fresh liver extract from lamb as described earlier by us<sup>4</sup> was used. The distribution of corpuscles was estimated from the last ten proglottids excluding the end-proglottid of worms stained with silver nitrate solution<sup>5</sup>. The significance of difference, as compared to the control was determined<sup>6</sup> by student's *t* test.

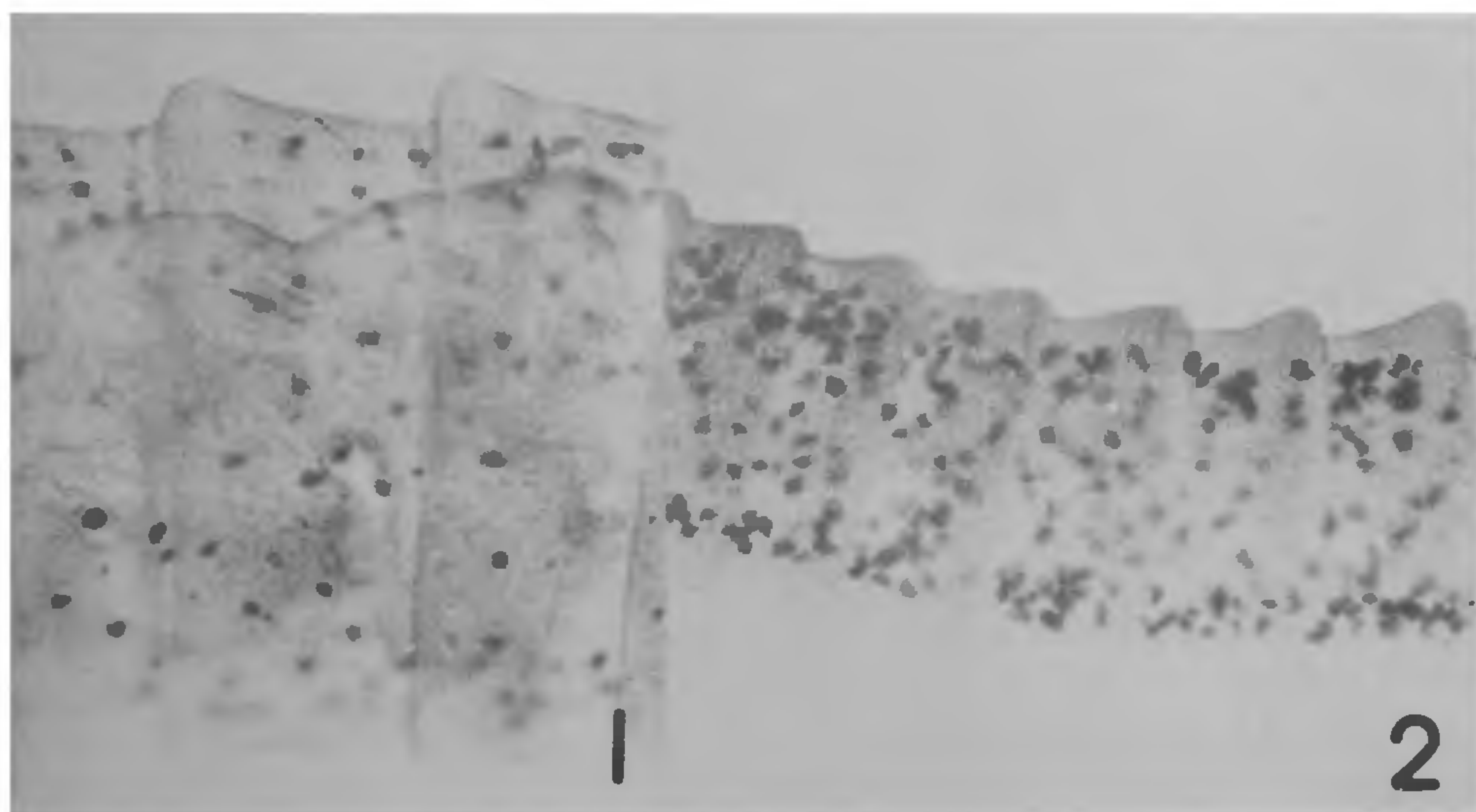
There is a significant ( $P < 0.01$ ) increase in the number of corpuscles in the proglottids of worms grown in media containing bile as compared to those grown in the absence of bile. The number of corpuscles gradually increased in the proglottids in the medium containing upto 0.12% of ox bile. However,

**Table 1** Effect of different concentrations of ox bile on the numbers (mean  $\pm$  SE) of calcareous corpuscles of 11-day-old *Hymenolepis microstoma*, grown in vitro from 4-11 days.

Concentration (%) of ox bile in medium					
No bile	0.015	0.06	0.12	0.30	0.60
46.84 $\pm$ 2.43 (11.6)	54.86 $\pm$ 2.98 (12.2)	62.90 $\pm$ 3.51** (12.5)	66.98 $\pm$ 3.87** (12.2)	17.42 $\pm$ 1.10** (14.1)	1.46 $\pm$ 0.25** (38.3)

Each figure is the average of determinations on 50 proglottids. Figures in paranthesis indicate the coefficient of variation.

\*\*  $P > 0.01$



**Figures 1, 2.1.** Whole mount of 11-day-old *H. microstoma* (middle third) grown in the medium without ox bile ( $\times 140$ ). **2.** An 11-day-old worm grown in medium containing 0.12% ox bile showing difference in the size of the proglottids and number of corpuscles ( $\times 140$ ).

at higher concentration of ox bile the number of corpuscles was decreased drastically (table 1). The difference in the distribution of corpuscles in the proglottids of worms grown in the medium containing 0.12% ox bile and that without the bile is further evident from figures 1 and 2. At 0.30% or 0.60% concentration, the constituents of bile may have a lytic effect indirectly affecting the formation and numbers of calcareous structures.

The calcareous corpuscles are known to have  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  and  $\text{PO}_4^{3-}$  as their principal components<sup>7</sup>. It is, therefore, conjectured that ox bile may increase the formation of calcareous corpuscles *in vivo*, possibly by increasing the permeability of the membranes. How-

ever, the mechanism of action of bile in modifying the uptake of nutrients is yet to be explained. From the present studies, it is obvious that the formation of corpuscles, at least in *H. microstoma*, are maximum at 0.12% concentration of bile.

The first author (NC) is grateful to the Belgian Ministry of Education (International Cultural Relations) for a fellowship.

2 July 1984; Revised 17 September 1984

1. Smyth, J. D., *The physiology of cestodes*, Oliver and Boyd, Edinburg, 1966, p. 3.



2. Chowdhury, N. and Rycke, P.H.de., *Jpn J. Parasitol.*, 1979, 28, 261.
3. Chowdhury, N. and Rycke, P.H.de., *Biol. Jb. Dodonaea.*, 1974, 42, 51.
4. Chowdhury, N. and Rycke, P.H.de., *Z. Parasitenk.*, 1978, 56, 29.
5. Chowdhury, N. and Rycke, P.H.de., *Z. Parasitenk.*, 1974, 43, 99.
6. Snedecor, W. G. and Cochrum, G. W., *Statistical Methods*, Oxford and IBH Publishing Co., Calcutta, 1976, p. 58.
7. von Brand, T., Nysten, M. U., Scott, D. B. and Martin, G. N., *Proc. Soc. Exp. Biol. Med.*, 1965, 120, 383.

### IMPROVED TECHNIQUE FOR CHROMOSOME STUDY IN SOME MEMBERS OF LABIATAE

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IMPROVED techniques were developed for the study of chromosome in some genera, such as *Ballota*, *Coleus*, *Lavandula*, *Mentha*, *Ocimum*, *Salvia* and *Scutellaria* of the family Labiatae. Three different mixtures containing  $\alpha$ -bromonaphthalene, aesculine and saponin, p-dichlorobenzene, aesculine and 8-hydroxy-quinolene and a third one containing only aesculine were found to be the most suitable combinations of pretreating chemicals in these genera.

The members of the family Labiatae, being aromatic, contain several types of essential oils in their cytoplasm. As a result, the difficulty in the study of their chromosomes, usually very small in size, has been strongly felt since long. The oil contents of the cytoplasm are considered to prevent the penetration of the pretreating chemicals to the cytoplasm and thereby keeping the viscosity balance between the cytoplasm and spindle mechanism and the metaphase chromosome system unaffected<sup>1</sup>. The various investigations, so far carried out in these species, have not been able to give the details of their chromosome structure. With this view in mind, different pretreating agents alone and in combinations were tried on 8 genera consisting of 21 species and 40 varieties and populations of Labiatae, collected from different parts of the world.

For the study of somatic chromosomes, several pretreating chemicals and their combinations were tried for different species and genera. Of these, a freshly prepared 1:1 mixture of  $\alpha$ -bromonaphthalene and aesculine with a slight addition of saponin was found to be the most successful in the species of *Ballota*, *Coleus*, *Lavandula*, *Mentha*, *Ocimum* and *Salvia*. A drop of  $\alpha$ -bromonaphthalene was taken in water and shaken well to prepare its homogeneous mixture. To this a bit of saponin was added and again shaken to complete the saponification. Mixture of aesculine was separately prepared by adding to water a little bit of aesculine powder with the help of a needle to produce just a blue tinge. These two mixtures were then mixed in a 1:1 ratio. Pretreatment was then done with fresh and healthy root tips kept in this mixture and chilled for 3 min at 4°C. After cold treatment, the pretreating mixture with root-tips were kept at 14–16°C for 1 to 2 hr depending upon the number of chromosome in the different species<sup>2</sup>. However, to get the desired results in *O. gratissimum* in the saturated aqueous solution of p-dichlorobenzene, a trace of aesculine was added in the manner described before and then 2 drops of 8-hydroxyquinolene was mixed. The mixture of these pretreating agents with root-tips were kept at 4°C for 5 min and then transferred to 16°C for 2 hr. In the various species of *Scutellaria*, satisfactory results were obtained in an aqueous mixture of aesculine only<sup>3</sup> (table 1.) All the mixtures were prepared fresh and in tap water.

During the course of this study, certain interesting observations were made. It was found that the aqueous mixture of  $\alpha$ -bromonaphthalene, aesculine and saponin was successful in 32 varieties and populations under 15 species and 7 genera. All the species and populations of *Ocimum* responded to this mixture alike, except the 2 populations of *O. gratissimum* (table). In all these cases, good metaphase plates with fine clarity of chromosomes were obtained. Despite their small size, primary and secondary constructions and satellites were clearly observed in the chromosomes and they were counted with great ease due to their fine scattering in the semisolid cytoplasmic background.

In the species with high essential oil contents (*Ocimum*, *Mentha*), saponin comparatively acted well. It can be suggested that saponin clears the cytoplasm, thereby facilitating the penetration of the pretreating chemicals.

Almost all the species under investigation showed that they needed the duration of pretreatment according to their chromatin contents or chromosome num-