

**Table 2** Percentage mortality of larvae and adults of *Calopepla leayana* treated with spore suspension of *Beauveria bassiana*

Treatment	Spore concentration	Percentage mortality		
		After 5 days		After 11 days
		Larvae	Adults	Adults
Direct application of spore suspension	$1 \times 10^5$	33	85	100
Spores applied on leaves	$1 \times 10^5$	30	80	100
Control	Sterile water	5	8	11

ranged between 20 and 33°C and RH 65 and 96%). The reason for higher mortality of adults in comparison with larvae of *C. leayana* is not clear.

*B. bassiana* is a known pathogen of many insects in both temperate and tropical countries<sup>3-5</sup>. The fungus is considered to be potentially suitable for biological control of insects due to its broad spectrum of virulence to insects and its suitability for large scale culture<sup>6,7</sup>. *B. bassiana* is best known as the causal agent of the disastrous muscardine in silkworms<sup>8</sup>. In teak, it is reported to cause mortality of leaf skeletonizer, *E. machaeralis* in Karnataka, India<sup>9</sup>. The present study reports the potentials of *B. bassiana* as a biocontrol agent against *M. viridanus* and *C. leayana* under laboratory conditions. Further studies are, however, necessary to find out its potential as an agent of biocontrol against these pests under field conditions.

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## INFLUENCE OF METHOPRENE ON THE MALE REPRODUCTIVE SYSTEM OF *ORYCTES RHINOCEROS* (COLEOPTERA: SCARABAEIDAE)

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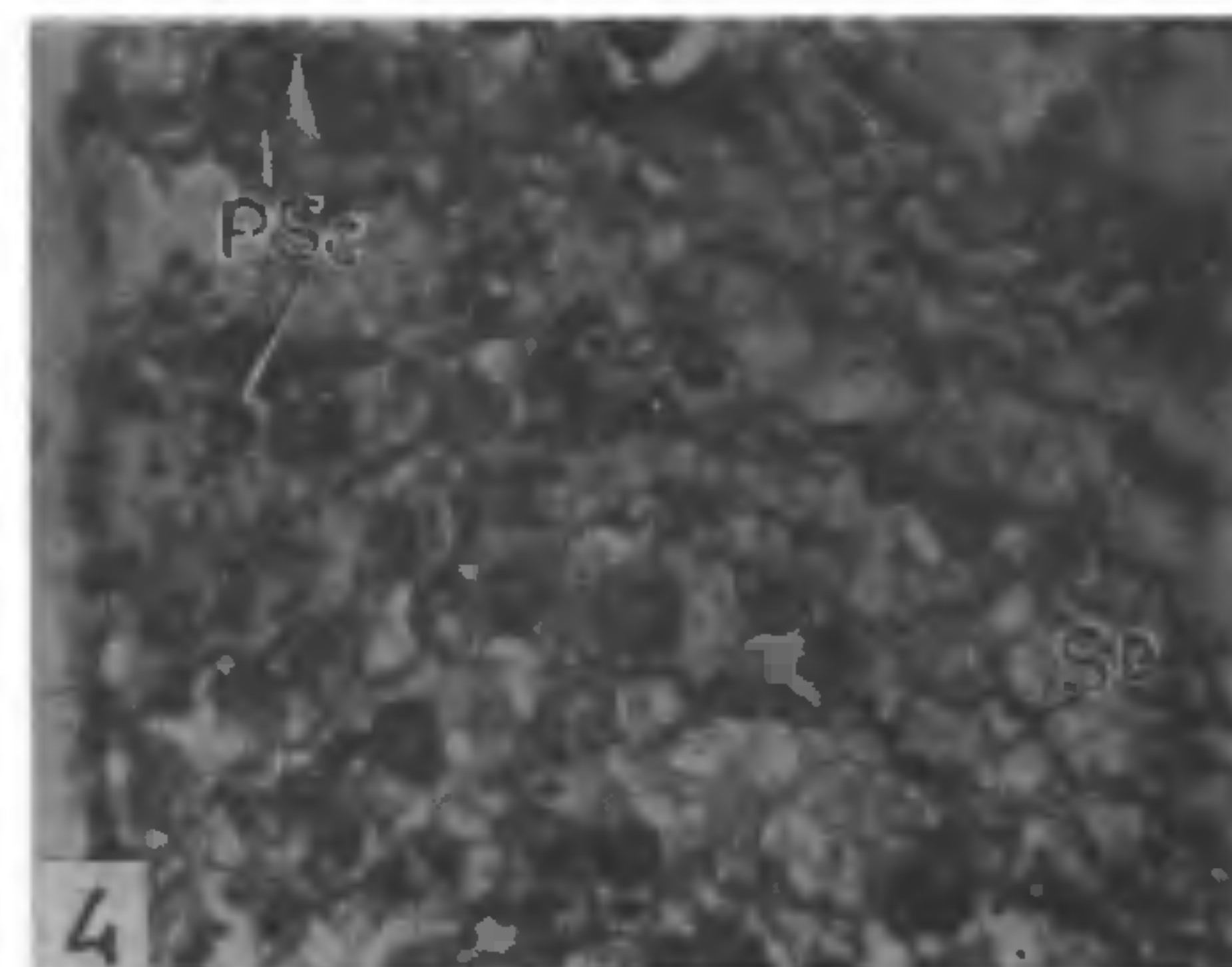
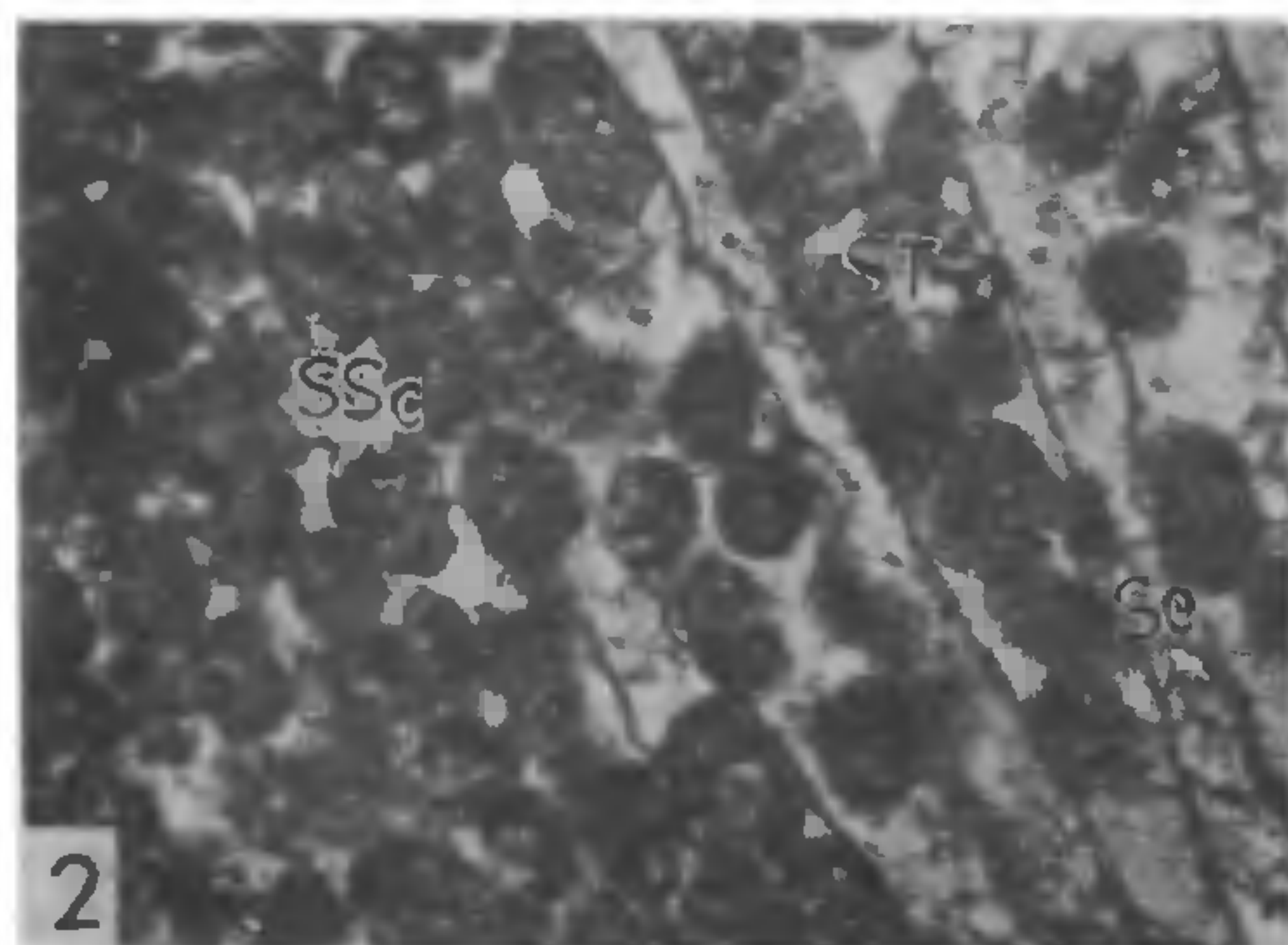
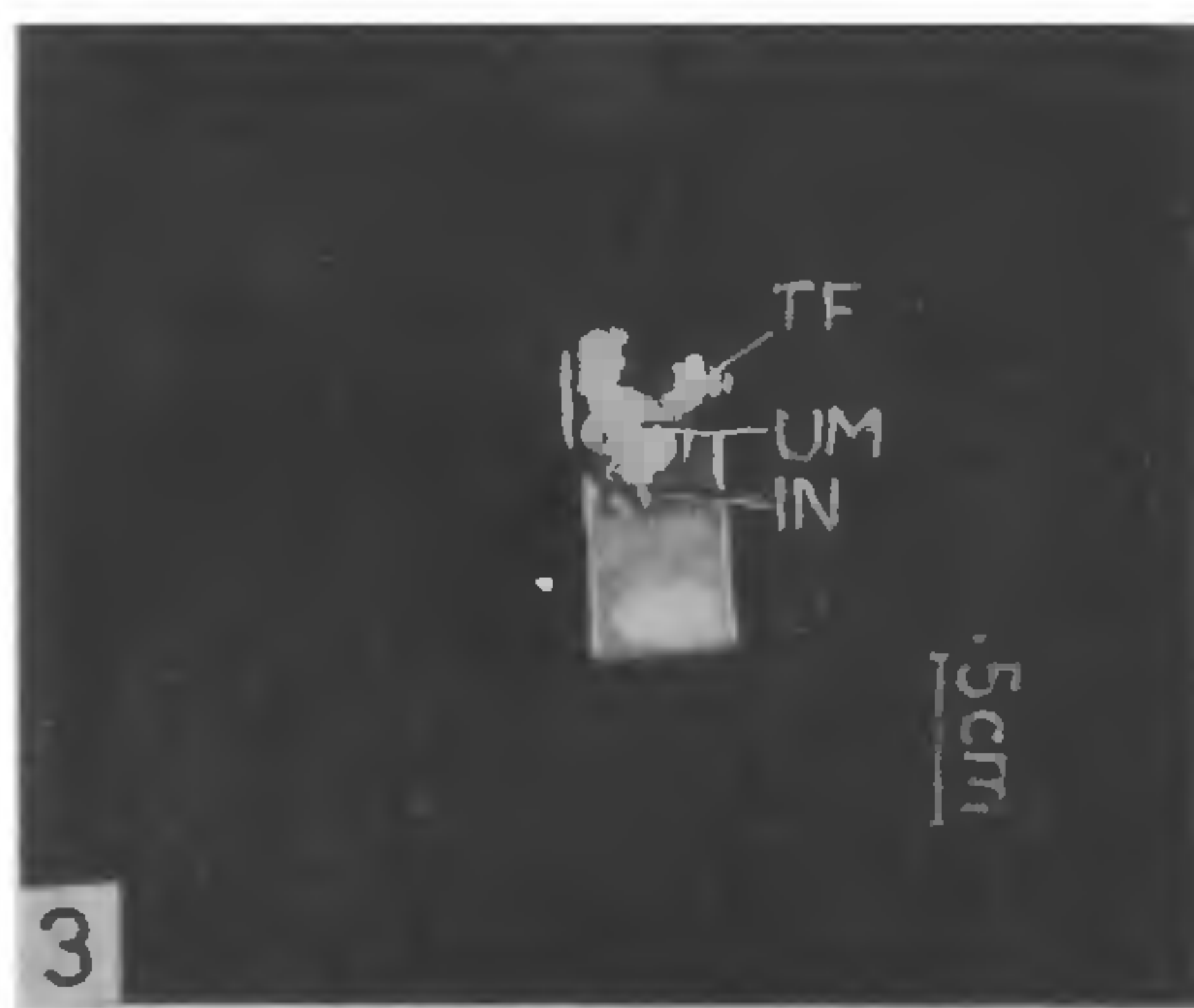
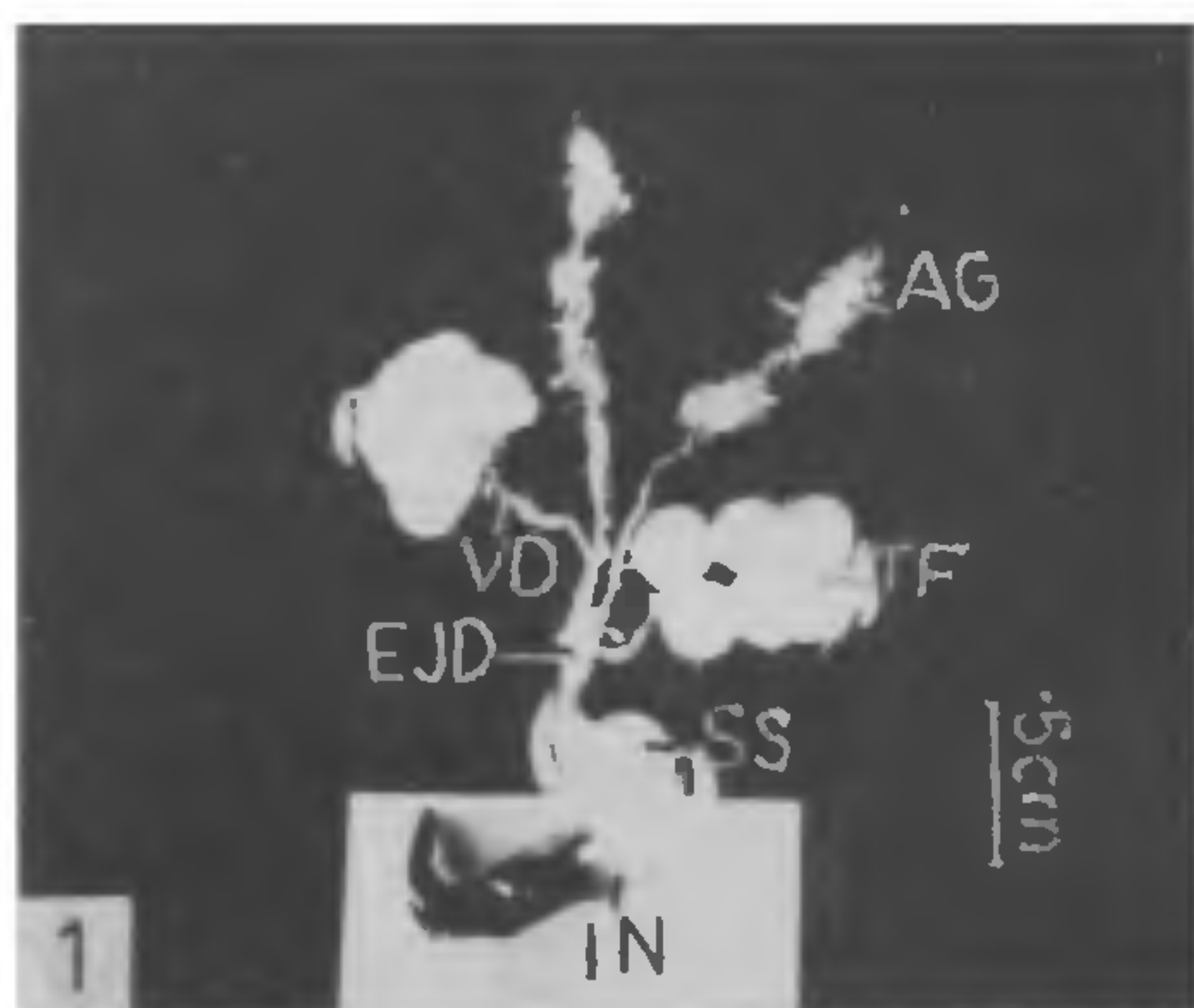
SPERMATOGENESIS in insects is believed to be under hormonal control<sup>1</sup>. Dhondt *et al*<sup>2</sup> found that methoprene treatment resulted in 'adultoids' (pupal-adult intermediates) in rhinoceros beetles; the authors however overlooked their internal structure. The present study gives details of action of methoprene on the male reproductive system of *Oryctes rhinoceros*, a major pest of the coconut palm.

Third instar larvae of *O. rhinoceros* were collected from local manure pits and reared in the laboratory on sterilized cowdung in small plastic containers. Methoprene (ZR 515, gift from Dr G. B. Stall of Zoecon Corporation, USA) was dissolved in acetone so that 1 µl contained the desired quantity of hormone (100, 50 and 20 µg). The hormone was applied topically on the ventral abdominal segments of newly emerged male pupae. The controls received only acetone treatment.

From the control pupae normal adults emerged with well-developed reproductive system from 16 to 18 days of pupation. In these the testis follicles (six in number) were separate disc-shaped organs, each follicle measuring 1-2 mm diameter (figure 1). In each follicle secondary spermatocysts and early

spermatids were arranged in the compartments formed by intra-follicular septa (figure 2). Methoprene-treated pupae resulted in adultoids which lived for six to eight days and were characterized by adult head, pupal wing buds, pupal abdomen and protruded intromittent organ. A dose-dependent effect was seen with regard to differentiation of reproductive system. In the 100  $\mu$ g treated pupae the testis retained the structure of zero day normal pupa showing that the development was arrested during topical application. The small testes were attached to an undifferentiated mass of tissue with chitinized paired tips representing intromittent organ, without

any differentiation of ejaculatory duct, vasa efferentia, vasa deferentia or accessory glands (figure 3). Primary and secondary spermatogonia observed in a zero day pupa remained as such without any further development. In the 50  $\mu$ g applied pupae the testis follicles were enlarged in size but the rest of the system remained as observed in 100  $\mu$ g treated pupae. A reduced number of primary spermatocytes were seen in the testis follicles and they were scattered in the compartments formed by septa. The septa were thicker and multi-layered when compared to that of controls (figure 4). When 20  $\mu$ g was applied the testis follicles reached almost the normal



**Figures 1–4.** 1. Male reproductive system of control 0-day adult; 2. TS through testis follicle of 0-day-old adult control ( $\times 400$ ); 3. Male reproductive system of 100  $\mu$ g methoprene-treated adultoid at emergence, and 4. TS through testis follicle of 50  $\mu$ g methoprene-treated adultoid ( $\times 400$ ). [AG, accessory gland; EJD, ejaculatory duct; IN, intromittent organ; PSc, primary spermatocyte; Se, septa; SSc, secondary spermatocysts; ST, spermatids; TF, testis follicles; UM, undifferentiated mass; VD, vas deferens.]



size but the ducts and intromittent organ were rudimentary. The accessory glands were represented by small ducts without glandular portion. Secondary spermatocysts became evident in the follicles but they were lesser in number and were crowded among the spermatogonia.

Dhondt *et al*<sup>2</sup> reported adultoids in rhinoceros beetles as a result of JH analogue treatment. The present study reveals that the differentiation of male reproductive system in adultoids of *O. rhinoceros* is inhibited as a result of methoprene treatment. Methoprene applied to early pupae of *O. rhinoceros* showed a dose-dependent inhibition of male reproductive system and sperm differentiation. The inhibitory action of JH analogues on the male reproductive system has been reported in *Trogoderma granarium*<sup>3</sup>, *Spodoptera littoralis*<sup>4</sup>, and *Scirpophaga incertulas*<sup>5</sup>. When the dose applied was reduced in *O. rhinoceros* a reduced number of differentiating spermatocytes were seen as reported in *Corcyra cephalonica*<sup>6</sup>. In *O. rhinoceros* methoprene also inhibited the development and differentiation of male accessory glands.

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## EFFECT OF PENICILLIC ACID ON ISOLATED FROG HEART

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PENICILLIC acid, an  $\alpha,\beta$ -unsaturated conjugated lactone is produced by various food-borne fungi<sup>1</sup>. Earlier investigations revealed that penicillic acid is carcinogenic in rats and mice<sup>2</sup>, cytotoxic in cultured cells<sup>3</sup> and hepatotoxic in mice<sup>4</sup>. Penicillic acid effects on erythrocytes<sup>5</sup> and on intestinal brush border of rabbits<sup>6</sup> have been reported recently. Murnaghan<sup>7</sup> reported that penicillic acid has a digitalis-like effect on cardiac muscle. Digitalis increases the force of contraction (positive inotropic action) but slows the ventricular rate<sup>8</sup>. Based on this, the present work was undertaken to investigate the effect of penicillic acid on heart tissue using isolated frog's heart as the experimental model.

Isolation and purification of penicillic acid have been reported earlier<sup>6</sup>. Frogs (*Rana hexadactyla*) with a live weight of 100–120 g were used for isolation and perfusion of heart.

Heart was perfused with Ringer's solution through a cannula tied into the inferior vena cava, the cannula was connected to perfusion funnel and the funnel was opened into the cannula. The height of the funnel and the cannula was adjusted so that the fluid remained in the vertical limb of the cannula to about three-fourths of its height. The apex of the heart was hooked to a recording lever. The rhythmic beating of the heart was recorded using a smoked drum of a kymograph<sup>9</sup>.

To study the effects of penicillic acid at various levels, different concentrations (1 mg/ml, 1.5 mg/ml, and 2 mg/ml) were prepared in Ringer's solution.

Isolated heart was perfused, first with Ringer's solution and the normal recording done<sup>9</sup>. This was followed by perfusion with various concentrations of penicillic acid as mentioned above. The effect of the perfusate was recorded systematically on the kymograph. In the present study, six isolated frogs' hearts were used and recordings from the six frogs showed consistent results. The figure shows one such recording.

Murnaghan<sup>7</sup> reported that penicillic acid in concentrations of  $10^{-3}$  g/ml and  $10^{-4}$  g/ml exerted a digitalis-like effect on cardiac tissue. The same author substantiated this by noting that penicillic