ppm/20 gm. Each batch consisting of 50 insects in triplicate was kept in the plastic container along with the treated medium at an isolated place in the laboratory for observations. Simultaneously a similar batch was maintained on wheat flour treated with acetone solution of JHA. Monthly observations were made during which the dead insects, dried larvae and pupae as well as exuviae were removed while keeping the remaining insects in the same medium. For about seven months the supernumerary larvae were found active and finally the mortality count of the larvae was made after six months.

The larvae that emerged from the eggs laid by the insects exposed to the treated food passed through the normal development until the last instar larva and a majority of them moulted into pupae and adults. A few larvae were noticed not entering into pupation and in the subsequent observations till the seventh month the size of these larvae increased considerably and moulting into extra larval instars more than once. During the later stages these supernumerary larvae were cylindrical in shape and considerably increased body size (0.9 to 1.0 cm as compared to the normal size of 0.4 to 0.6 cm) with hard segmental tergum and striped inter-segmental membrane (figure 1). Head capsule and thorax were heavily chitinized and enlarged setae all over the body. At this stage these larvae looked totally different from the normal last instar larvae of *Tribolium*. They were very active with periodical shedding of cuticle but there was no pupation. Stoppage of further growth and cessation of feeding was observed.

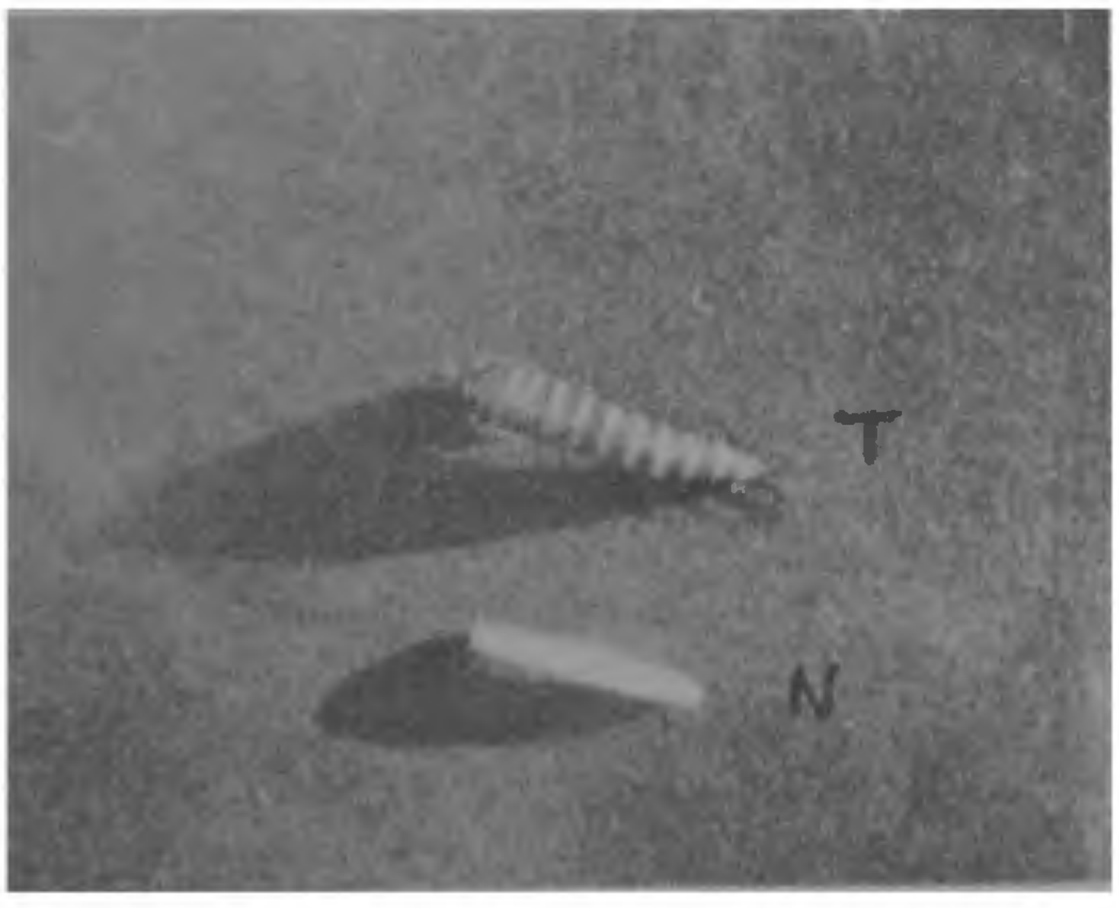

Figure 1. Supernumerary 5th instar larva of *T. castaneum* after the treatment of Kinoprene ZR 777. T-Treated, N-Normal.

Earlier reports showed juvenoids causing delay of pupation or puparium formation when treated to the last larval instars of certain endopterygotes including the Coleoptera[3]. Delay of pupation in these insects was also correlated with the dose of JHA applied. It was assumed by Slama[2] that there is an incomplete breakdown or excretion of the endogenous corpora allata hormone in the young larval stages due to which, development of last larval instar into pupae was suspended as long as JH remained in the body. Therefore, some of the larvae remaining as supernumerary larval forms for a prolonged period may be attributed to the above contention besides the constant presence of JHA applied in the food medium. However what is interesting in the present observations is the formation of supernumerary larval moult with the treatment of JHA orally to the adult insects which normally does not take place in Coleoptera when last larval instar is exposed to the juvenoid[2]. This interesting findings, suggesting the possibility of forming extra-supernumerary moults besides delay in the pupation, merit further investigations.

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1. Suchy, M., Slama, K. and Sorm, F., *Science.*, 1968, **162,** 582.
2. Slama, K., *Insect hormones and bioanalogues.*, (eds) K. Slama, M. Romanuk., F. Sorm., Springer-Verlag, New York. 1973, pp.111, 223.
3. Slama, K., *Annu. Rev. Biochem.*, 1971, **40,** 1079.

AN ASSESSMENT OF THE MOSQUITO-PATHOGENIC FUNGUS *LEPTOLEGNIA* (SC-I) AS A BLACKFLY (DIPTERA: SINULIIDAE) PATROGEN

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SEYMOUR[1] reported the isolation of a water mold from a parasitized mosquito larvae which he tentatively identified as *Leptolegnia*; however, he attempted no further experiments with artificial infection. Mortality

due to Leptolegnia (Ohio isolate) infection, has been demonstrated in Geratopogonidae; *Culicoides* sp, Chironomidae: *Chironomus* sp; Chaoboridae: *Mochlonyx* sp; however, it is not known to infect crustacea, Amphipoda, Cyclopoidae, Coppepods, amphipods and planaria (Dr. Berry pers. Comm.). It has been observed to infect and kill 100% second instar larvae of *Culex pipiens pipiens Anopheles quadrimaculatus, Aedes triseriatus, Culex restuans, Haemogogus equinus,* and *Toxorhynchites rutilus* (pers. observations).

In 1975, *mosquito* larvae collected in South Carolina were infected with a fungus similar, if not identical to the *Leptolegnia* reported earlier[2]. It has been reported to be highly virulent for early instar larvae of *Culex, Aedes, Anopheles, Culiseta, Psorophora* and *uranctaenia*[3-5].

The present note reports that the South Carolina isolate is not markedly pathogenic to the blackflies.

The South Carolina isolate of *Leptolegnia* (SC-I) was used in all experiments. The fungus was grown on Z-medium (I part hemp seeds extract, yeast glucose to I part wheat germ yeast glucose) on a water bath shaker for 24 hr at 28°C. Mycelia were collected, washed, zoospores produced and counted on a haemocytometer for assay.

Field-collected larvae of *Simulium neavei* and *S. damnosum* were rinsed with 5% sodium hypochlorite for 2 min and then placed in glass beakers (100 larvae/replicate) containing 1 litre of vigorously aerated stream water at 20–24°C and allowed to acclimate 24 hr before treatment. Control replicates were treated with heat killed zoospores. Concurrent assays were conducted with mosquito larvae as a check for fungal pathogenicity. In these tests 2nd instar *Aedes aegypti* L. larvae were treated in glass beakers holding 100 ml of sterile distilled water with 2 or 4 replicates of 25 larvae per dose.

Exposures were ended by flushing the bioassay units with stream water (black flies or distilled water (mosquitoes). Inoculum settling from suspension in black fly experiments was resuspended at least once daily. Larval mortality was recorded after 8 days.

Despite treatment at a concentration as high as 4×10^5 zoospores per ml (3 days exposure) only 23.5% mortality occurred in both *S. neavei* and *S. damnosum*. Exposures for as long as 6 days (4×10^6 zoospores/ml) caused only 21.5% mortality in *S. neavei* and 20.5% mortality in *S. damnosum* respectively. In contrast, 100% mortality of *A. aegypti* larvae was observed at a dosage of 2.4×10^4 zoospores/ml (24 hr exposure) or about 1/160 of the highest concentration tested for black flies. These results confirm the sevcre pathogenicity of this isolate of *Leptolegnia* sp (SC-I) to mosquitoes; however, the reason for its apparent lack of pathogenicity against black flies is not clear. Dissections showed that zoospores were readily ingested, completely packing the black fly gut throughout the exposure period, but germ tube penetration into the haemocoels of larvae was not observed. Factors that might augment infection (temperatures, larval age, dosage, zoospores viability) were highly favourable for infection and therefore the cause of failure of infect on black flies is unknown.

It is clear that the South Carolina isolate is not markedly pathogenic to black flies, and its use as a black fly larvicide cannot be recommended. Even if a pathogenic species of this fungus were found and used, black fly control might be impractical because of the long exposure times necessary in the lotic habitat of the insect.

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1. Seymour, R., In *Biological regulation of vectors; The saprophytic and aerobic bacteria and fungi.* (ed.) D. John, Briggs, IHEW Pub. No. (NIH) 77–1180. U.S. Govt. Printing Office, Washington, D.C. 1976, pp. 135.
2. Muenleisen, D. P., M. S. thesis 1977, Clemson University.
3. Sweeney, A. W., In *Biocontrol of medical and veterinary pests* (ed.) M. Laird, 1981a, pp 105, Praeger Scientific Publishers.
4. McInnis, T. Jr. and Zattan, W. C., *J. Invertebr. Pathol.*, 1982 **39**, 98.
5. Hall, R. A. and Papierok, B., *Parasitology*, 1982, **84**, 205.