# Fecundity of *Panagrellus silusiae* Treated with Ethyl methanesulphonate and 5-bromouracil[1]

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Techniques developed for synchronous growth experiments (6, 8, 9) on *Panagrellus silusiae* (de Man) Goodey were used to test the effects of two chemical mutagens, 5-bromouracil (5-BU) and ethyl methane sulfonate (EMS) on the fecundity of that nematode.

Mixed stages of *P. silusiae* grown on Czapek Dox agar were exposed to 0.2, 0.5, 0.75 and 1% EMS for 8 hr at 25 C to determine its toxicity. Second stage larvae survived only in 0.2% EMS; all other stages survived in all concentrations tested.

Second stage larvae were isolated from a population of mixed stages by passage through a 125 ml separatory funnel containing 0.5 mm diameter glass microbeads (6, 8, 9). Approximately 300 animals were placed in 0.25 ml of water over 0.25 ml Nigon's agar (5) in each of a series of depression slides, and their growth monitored as previously described (6, 8, 9). Every 8 hr for 64 hr, samples from these cultures were transferred to 0.2% EMS in physiological saline (PS), 0.2% 5-BU in PS, or PS alone (control) and incubated 8 hr at 25 C. Treated nematodes were washed six times in PS and six depression slides with water over Nigon's agar containing 10 animals each were prepared from each treatment. The number of progeny of the 126 depressions so set up (eight different times with six replicate depression slides for each of three treatments per time) were counted 140 hr after the initial isolation of second stage larvae (Table I).

The remaining worms from each time and treatment were grown in depressions containing water over Nigon's agar and gonad development was followed by staining samples at 8-hr intervals with proprionic orcein (4). A 1:1 sex ratio was confirmed. No mortality of developing stages was observed but some mature females died of *endotokia matricida.*

In view of the differing modes of action of EMS and 5-BU [EMS by alkylation of purines (3) and 5-BU by promotion of base-pair transitions (1), the former not requiring DNA replication for mutagenesis] the similarities of their effects upon *P. silusiae* fecundity was surprising. EMS was more active (and even fatal to nematodes in the 350-$\mu$ range) than 5-BU but both were most active at similar stages of development.

Although no visible cytological differences were detected in genital primordia of stained L2–L4 juveniles, early mutagenesis is most likely. In L2's the genital system consists of only four cells that begin to proliferate only after the second moult (2, 7). Chemical disturbance of chromosome replication or kinesis of one of these would be most likely to produce an abnormal reproductive system.

Another possibility, supported by the fecundity data (Table I) is that maximum mutagen effect occurred during the L2 and L3 moults at 450 and 600 $\mu$ (7). The cuticle of L2 juveniles is thinner (7, 8) and increased permeability of cuticle during ecdysis has been shown (9).

Even though decreased fecundity may have resulted from direct toxicity, several morpho-

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TABLE 1. Effects of 8-hr, 0.2% ethyl methanesulfonate (EMS) and 5-bromouracil (5-BU) treatment upon subsequent fecundity of *Panagrellus silusiae* juveniles treated at controlled levels of development.

| Mean Length at Treatment ($\mu$) | Life Cycle Stage | CONTROL No. of Progeny | 5-BU | | EMS | |
|---|---|---|---|---|---|---|
| | | | No. of Progeny | % of Control | No. of Progeny | % of Control |
| 280 ± 3[a] | L2 | 2311 | 807 | 35.2 | 384 | 16.8 |
| 350 ± 7 | L2 | 2538 | 594 | 23.4 | [b] | — |
| 453 ± 7 | L2–L3 | 2382 | 572 | 23.2 | 83 | 3.5 |
| 551 ± 11 | L3 | 2843 | 1743 | 61.1 | 1437 | 50.5 |
| 646 ± 10 | L3–L4 | 2712 | 1422 | 52.5 | 426 | 15.7 |
| 754 ± 11 | L4 | 2640 | 1765 | 66.9 | 1314 | 49.9 |
| 767 ± 20 | L4 | 2509 | 1817 | 72.5 | 1278 | 51.0 |

[a] Standard error.
[b] Treatment fatal.

logically abnormal *P. silusiae* populations were isolated from mutagen-treated groups, which proved that mutagenesis occurred. Genetic investigation of these lines is being continued and will be reported later.

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