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Increased sensitivity to a natural pyrethrum extract of *Trypanosoma*-infected *Glossina morsitans*

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Summary

Topical application of a natural pyrethrum extract on male and pregnant female *Glossina morsitans morsitans* resulted in higher mortality for flies infected with *Trypanosoma brucei brucei* than for uninfected control flies. Infected males showed a significantly higher mortality while infected pregnant females showed a marginally significant increase in mortality. Results support the hypothesis that infected flies are less healthy than uninfected flies. Results also parallel previous findings using endosulfan as the topical applicant and exclude the likelihood that the results were because of a peculiar effect of endosulfan.

Key words: *Glossina;* trypanosome; insecticide; pyrethrin.

Some biochemical, histochemical and behavioural changes have been reported to occur in *Glossina morsitans* when mature infections of *Trypanosoma brucei* are established in the salivary gland (Golder and Patel, 1980; Patel et al., 1982; Jenni et al., 1980). Jenni and colleagues (1980) reported increased probing frequency and increased voracity of feeding in infected flies. These and other observations led us to hypothesize that the infected fly is stressed and likely to be less healthy than non-infected flies. We tested this contention by comparing mortality of infected versus uninfected flies when treated with low doses (LD_{50} or less) of endosulfan (Golder et al., 1980). We found that infected flies were significantly more sensitive to the insecticide than were uninfected flies. We considered the results to support our hypothesis. To rule out the possibility that the increased mortality may have been the result of some peculiar effect of endosulfan, we extended our experiments to include a natural pyrethrum extract. Pyrethrins are well known for their insecticidal properties.

The flies used in this study, *Glossina morsitans morsitans* Westwood, were reared in our insectary. Within 24 h of emergence, flies were fed on a rat at first

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peak of parasitemia, infected with *Trypanosoma (Trypanozoon) brucei brucei* (E.A.T.R.O., 1969). Female flies were mated on day four. Saliva was collected from the flies three to four weeks after the infected meal and examined for the presence of parasites. Infected flies were segregated and placed in numbered plastic holding tubes with mosquito netting on the ends. Periodic examination of the saliva allowed us to monitor the course of the infection. Uninfected flies served as the control group. All flies were fed daily on rabbits (except Sunday) and kept at 25° C and 80% relative humidity.

Infected and control flies, at 40–45 days of age, were dosed with a natural pyrethrum extract consisting of 60.21% pure pyrethrins I and II (Pythrethrin I = 35.52%, Pyrethrin II = 24.69%). The extract was diluted to 1 mg/ml in acetone to serve as a stock solution and kept at –20° C until use. Working solutions for topical application were diluted with acetone and used immediately. One microliter of pyrethrum extract was applied to the fly's dorsal thorax with a microapplicator (ISCO, Model M, Instrumentation Specialities Co. Lincoln, Nebraska, USA) fitted with a 0.25 ml glass syringe and a 27 gauge neddle. Flies were hand-held while dorsing without anesthesia. The difficulty in obtaining large numbers of infected flies prohibited us from performing a complete probit regression analysis of mortality between the infected and uninfected groups. We therefore chose a low dose (below LD_{50}) to discriminate between the two groups. A trial experiment on uninfected flies suggested that a dose of 0.5 ng/fly for the males and 3 ng/fly for the females should give a low enough mortality in the controls to discriminate between the two groups. Mortality was recorded at 48 h. Dead flies were dissected to verify the presence or absence of infection.

Results are summarised in Table 1. Chi square analysis shows that there is a significant difference in mortality of infected males versus non-infected males $\chi^2 = 16.041$ ($p < 0.001$). For pregnant females the significance level is considerably less, $\chi^2 = 3.527$ ($0.05 < p < 0.10$).

In a previous study we found significant differences in mortality between infected and uninfected flies of both sexes when treated with low levels of endosulfan (Golder et al., 1982). The results of this study are similar in that increased mortality of infected versus uninfected males is highly significant while the increase in mortality of infected versus uninfected pregnant females is less. Recent studies provide some insight as to why the increase in mortality in infected versus uninfected pregnant, female, flies shows a lower level of significance than do the males (Kwan et al., 1982). They showed that females acquire higher tolerance to endosulfan during the pregnancy cycle. Increased tolerance levels they reported were: 3-fold after 48 h, 8-fold at mid-cycle (4–5 days) and from 10- to 14-fold at 24–28 h prior to larviposition. Hadaway (1972) also has reported increased tolerance to resmethrin (a synthetic pyrethrin) in pregnant female tsetse flies. We known that 40–45-day-old flies, reared in our insectary and mated on day 4, would be in the middle or late in their third cycle, or early to mid-way in the fourth cycle of pregnancy. Thus, both the infected group and the

Table 1. Mortality at 48 h after topical application of a natural pyrethrin extract

Flies	Dosage	No. dosed	No. dead at 48 h	Percent mortality
Non-infected males	0.5 ng/fly	101	14	14%
Infected males	0.5 ng/fly	26	13	50%
Non-infected females	3 ng/fly	175	70	40%
Infected females	3 ng/fly	29	17	59%

Note the difference in mortality between infected and non-infected flies of both sexes. Chi-square values: males, $\chi^2 = 16.041$ ($p < 0.001$), df = 1; females, $\chi^2 = 3.527$ ($0.05 < p < 0.10$), df = 1

uninfected group of pregnant females would be expected to show an enormous variability in their pesticide tolerance level. This variability within each group would suggest that a large sample size would be needed to assess the significance level of increased mortality of infected versus uninfected pregnant, female flies. Nevertheless, the data as presented is statistically significant.

Our hypothesis that infected flies are not only less healthy but also more susceptible to pesticides is supported by *T. brucei*-infected *G. morsitans* showing increased sensitivity to a natural pyrethrum extract. Results of the present study also support previous findings (Golder et al., 1982) and confirmed that similar results using endosulfan were not likely to have been because of a peculiar action of endosulfan. Research on the physiology, pharmacology and behaviour of tsetse flies infected with other *Trypanosoma* species is needed to provide a data base for the design of an integrated tsetse control programme.

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