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Effects of carbon dioxide and nitrogen on *Glossina morsitans orientalis* Vanderplank

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The need for effective insect anaesthetics is indicated by the number of entomologists at present incorporating immobilizing agents into their research programmes. However, an increasing number of investigators have recently found that the anaesthetics produce undesirable side effects. For example, Harris *et al.* (1965) noted inconsistent results in toxicological investigations when they used carbon dioxide on stable flies, *Stomoxys calcitrans* (L.), and horn flies, *Haematobia irritans* (L.); also, prolonged inhibition of mating activity followed exposure of the cabbage looper moth, *Trichoplusia ni* (Hubner), to carbon dioxide or ether (Henneberry and Kishaba, 1967). Crystal (1967) summarized some of the adverse effects in insects resulting from the use of carbon dioxide and emphasized the need for critical analysis of the effects of anaesthetics used in entomology. This paper reports some effects of carbon dioxide and nitrogen on the length of life, reproduction, mating behaviour and feeding of *Glossina morsitans orientalis* Vanderplank.

MATERIALS AND CONDITIONS

For the test, we used adults that emerged in the laboratory from pupae collected in the Zambesi Valley near Kariba, Rhodesia. Emerging flies (0–24 hours old) were sexed and separated daily to ensure virginity, and the males and females were placed in separate 20 × 20 × 28 cm. cages (Dame and Ford, 1966), where they were maintained at a temperature of 26 ± 1°C. and a relative humidity of 70 ± 5 per cent. They were fed on guinea pigs each day for 45 minutes and were always offered food within three hours after narcosis with carbon dioxide or nitrogen. The caged flies were exposed to commercial carbon dioxide or nitrogen gas by placing the cages in a chamber large enough (20 × 25 × 33 cm.) to accommodate the fly cage. Gas was administered for three minutes at a rate of 15 litres per minute so that the chamber filled quickly and immobilization was rapid; then the gas flow was continued for the remainder of the exposure period at a rate of two litres per minute.

Sexually mature males and females (6–7 and 2–3 days old respectively) were used to investigate the effect of the treatment on insemination and reproduction. The rate of insemination was determined by dissection and microscopic examination of the spermathecae of surviving females. The reproductive capacity was determined by the number of pupae produced in 28 days. Rates of survival were obtained by recording the number of flies dying daily.

Length of Life

Single prolonged exposure. Groups of 80–200 newly-emerged adults (day 0) were ex-

posed in the treatment chamber for 1, 2, 3 or 4 hours. At the end of each time-interval, samples of 20–50 flies were removed and put into holding cages; the other flies, still in an immobilized state, were returned to the chamber for further treatment (the entire removal took about 30 seconds). The survival following the prolonged exposures to carbon dioxide or nitrogen is shown in figure 1. Since there were no statistical differences between the survival of treated and untreated groups, prolonged narcosis of newly-emerged adults did not affect length of life.

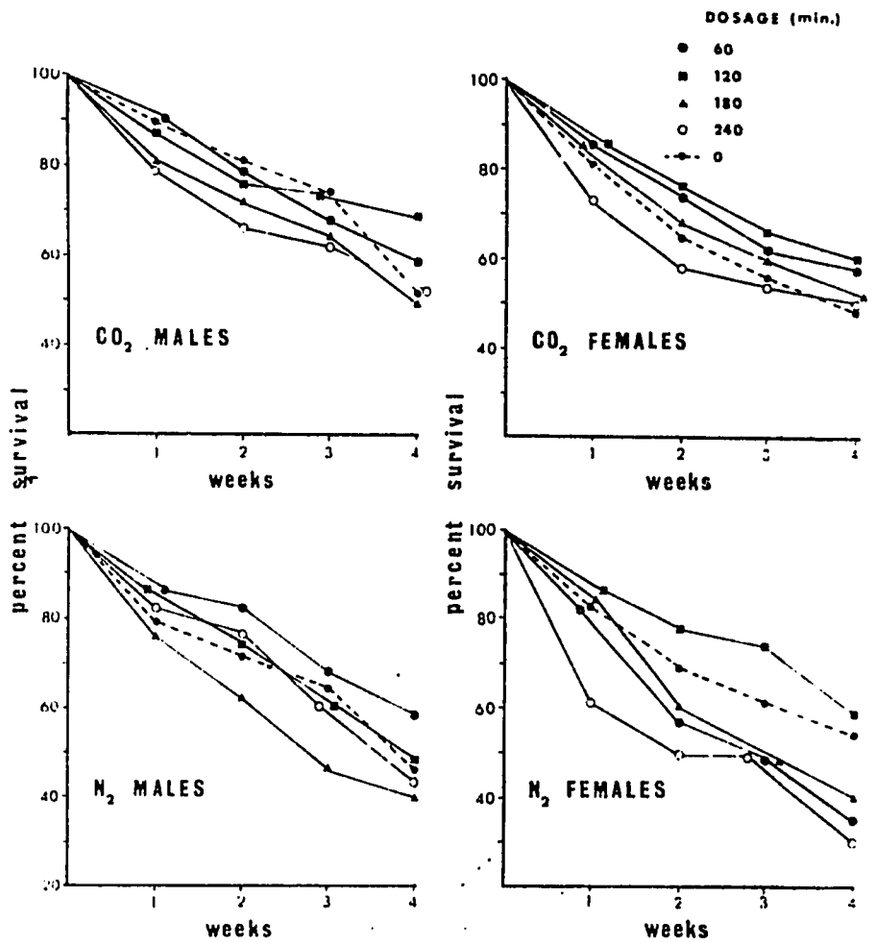

FIG. 1. Length of life of 20–50 adult *G. morsitans* after exposure to carbon dioxide or nitrogen when newly emerged (2–3 replicates). Treatment means are not significantly different from the control means at the 5 per cent. level of confidence.

Short and multiple exposures. The survival of flies exposed to carbon dioxide or nitrogen for 30 minutes as newly-emerged adults or as two-day-old adults, and the survival of adults exposed for 30 minutes each on day 0, 2, 4 and 6 post-emergence, are summarized in figure 2. Single exposures of newly-emerged (0-day-old) males and females to either gas

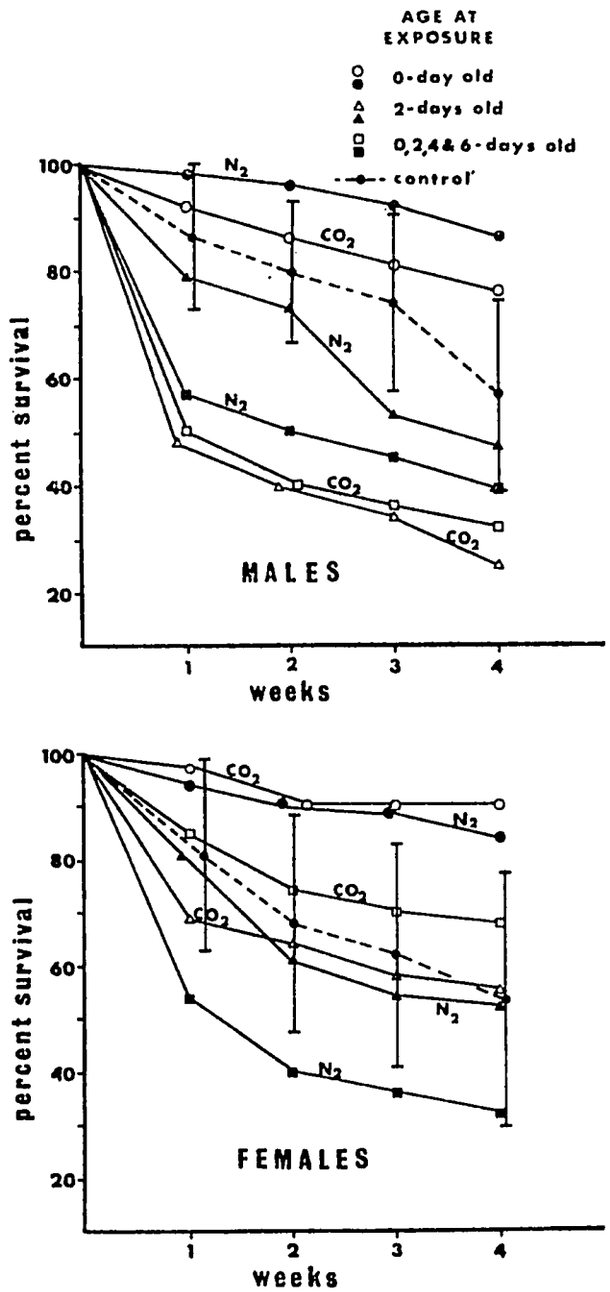

FIG. 2. Length of life of adult *G. morsitans* exposed to carbon dioxide or nitrogen for 30 minutes when newly emerged (0-day) or 2 days old, or for 30 minutes each on days 0, 2, 4, and 6 (2–7 replicates). The vertical lines indicate the 95 per cent. fiducial limits of the control means.

had no adverse effect; indeed, the survival of groups exposed once was significantly higher than that of the controls after four weeks. However, single exposures of two-day-old males to carbon dioxide caused a significantly reduced survival, though the survival of two-day-old males exposed to nitrogen and two-day-old females exposed to either nitrogen or carbon dioxide remained unaffected. Also, survival was significantly reduced when males were treated with multiple exposures to carbon dioxide though the females survived normally; similar exposures to nitrogen significantly reduced the survival of both sexes during the three immediately following weeks, but survival after four weeks was within normal limits.

In these tests, the survival of females was equal to or better than that of the untreated controls with the single exception of females treated with multiple exposures to nitrogen. Males exposed to carbon dioxide when they were two days old or exposed more than once had a reduced survival. Multiple exposures to nitrogen initially inhibited male survival, but after four weeks the survival was not significantly different from that of the controls.

TABLE I

Seasonal effect of a single 30-minute exposure to carbon dioxide or nitrogen on survival of 34–52 newly emerged male *G. morsitans* (2–4 replicates)

Treatment	Percentage surviving indicated week(s)			
	1	2	3	4
	Hot, dry season *October – November*			
Carbon dioxide*	85	78	70	63
Nitrogen*	92	85	78	71
Control	76	67	58	48
	Normal field conditions *March – June*			
Carbon dioxide*	92	86	81	76
Nitrogen*	98	96	92	86
Control	87	82	80	68

*Treatment means are not significantly different from control means at the 5 per cent. level of confidence.

Seasonal effect. We have observed (*unpublished data*) that the population of *G. morsitans* in the Zambesi Valley is exposed to severe environmental stress during the later months of the dry season (October–November). During these months, the mean weight of pupae collected in the field is less, and the resulting flies are generally smaller and less likely to survive than those emerging at other times of the year. We therefore compared the survival of males emerging in October–November with that of males emerging in March–June to determine whether a single 30-minute exposure to carbon dioxide or nitrogen would affect the survival of the hot-season adults differently. Table I shows that the survival of hot-season males was unaffected by exposure to carbon dioxide or nitrogen; their survival was equal to or better than that of the controls over the four weeks of observation. However, the survival of the untreated controls emerging in the hot season was 20 per cent. less than that of the untreated controls emerging in other seasons, and is indicative of the lower rate of survival obtained in the laboratory for flies emerging from pupae collected in the hot season.

Reproduction

The reproductive capacity of adults exposed to carbon dioxide or nitrogen for 30 minutes as newly-emerged males and females was studied by holding 24–25 sexually mature pairs together in a single cage for 28 days. At the end of the period, the survival and insemination of the untreated and treated groups did not differ significantly, and neither gas had a significant effect on reproduction (Table II).

TABLE II

Reproduction in 28 days by 24–25 pairs of adult *G. morsitans* after a single 30-minute exposure to carbon dioxide or nitrogen when newly emerged (2 replicates)

Treatment	Percentage surviving test ♂	Percentage surviving test ♀	Percentage insemination	Number of pupae
Carbon dioxide*	66	84	100	23
Nitrogen*	72	78	98	24
Control	80	70	100	22

*Treatment means are not significantly different from the control means at the 5 per cent. level of confidence.

Insemination

The effect of narcosis on male mating behaviour was determined by exposing the males to carbon dioxide on day 0 and again on the sixth day of life for four to five minutes—the time it takes to immobilize, count, and return them to a holding cage. Then immediately after the second exposure, the sexually mature males were caged with two-day old virgin females for 6, 24, or 48 hours. Microscopic examination of the spermathecae at the end of each period revealed that the insemination capability of the males was not affected significantly by the treatment (Table III).

TABLE III

Insemination of 20 females *G. morsitans* by 20 males exposed briefly to carbon dioxide on day 0 and again on day 6 before mating (2 replicates)

Treatment	Percentage insemination after indicated mating period (hours) 6	24	48
Carbon dioxide*	58	86	98
Control	60	92	92

*Treatment means are not significantly different from control means at the 5 per cent. level of confidence.

Feeding

Table IV shows the effects of a single four to five minute exposure to carbon dioxide on the feeding activity of newly-emerged males given an opportunity to feed within two hours after narcosis. Feeding rates were established by squashing the abdominal contents of each fly on filter paper and noting the presence or absence of blood. Carbon dioxide significantly inhibited feeding at two hours after treatment; however, after 24 hours, the feeding activity had returned to normal.

TABLE IV

Feeding behaviour of 12–20 male *G. morsitans* exposed briefly to carbon dioxide when newly emerged (5 replicates)

Day fed	Percentage fed Treated	Control
0	9*	46
0 and 1	67†	75

*Significantly different from the control at the 5 per cent. level of confidence.
†Not significantly different from the control.

DISCUSSION

Handling the flies was easier when carbon dioxide was used as the anaesthetic because it provided a more rapid knockdown and a slower recovery than nitrogen. However, it did cause bloating of the abdomen during treatment. This effect did not appear to be detrimental to the fly, and when the fly was removed from the anaesthetic, the slowly contracting abdomen provided a convenient criterion for judging when consciousness was about to be regained.

The reason for the significant increase in survival of newly-emerged males and females after exposure to carbon dioxide or nitrogen for 30 minutes is unknown. This effect did not occur with older flies or with those given prolonged exposures. Also, the better survival of females exposed to carbon dioxide after the day of emergence suggests that the female has a greater tolerance to carbon dioxide, but no sexual difference in tolerance to nitrogen was detected. In addition, we found a decreased tolerance with age similar to that demonstrated with screw-worm flies, *Cochliomyia hominiyorax* (Coquerel), treated with carbon dioxide (Crystal, 1967), but we did not find that tsetse flies became more resistant to narcosis by carbon dioxide as they grew older, as was noted with screw-worm flies (Crystal, 1967).

Exposure to carbon dioxide or nitrogen produced no apparent deleterious effect on reproduction or mating behaviour, and the insemination capability of male *G. morsitans* remained unaffected by four to five minute exposures to carbon dioxide. Thus, our results differ from those of Henneberry and Kishaba (1967) who found that short exposure of *Trichoplusia ni* to carbon dioxide caused reduced mating for 24 hours.

Feeding of the male tsetse fly was initially affected by carbon dioxide, but it returned to normal 24 hours after treatment. However, this temporary inhibition could be important in laboratory studies, e.g., those involving ingestion of parasites after narcosis. Also, in the field, it might affect the ability of flies to obtain a blood meal during the first few hours after release and would therefore diminish the chance of survival.

The evidence is sufficient to show that carbon dioxide and nitrogen are not entirely innocuous when they are used on tsetse flies. Both age and sex are apparently related to survival following narcosis, and other factors may also be involved. We suggest that workers ascertain the effect of narcosis in their individual situations before they adopt any technique as routine procedure.

SUMMARY

Adult *Glossina morsitans orientalis* Vanderplank emerging in the laboratory from pupae collected in the field were treated with single or multiple doses of carbon dioxide or nitrogen. Carbon dioxide produced a more rapid narcosis and slower recovery, but it did cause abdominal bloating (which was temporary and did not appear to affect survival). Single exposures of newly-emerged flies (0–24 hours old) to either gas for 30–240 minutes did not reduce length of life; flies exposed for 30 minutes had a slight, but statistically significant, increase in length of life. Reproductive capacity was not affected. Survival of two-day-old females exposed for 30 minutes to either gas was normal, as was survival of two-day-old males exposed to nitrogen, but exposure of two-day-old males to carbon dioxide resulted in a significant reduction in length of life. Exposure for 30 minutes each on day 0, 2, 4, and 6 to carbon dioxide significantly reduced male, but not female, survival; similar exposure to nitrogen reduced both male and female survival. The rate of insemination was not affected by exposure of newly-emerged males to carbon dioxide for four to five minutes on day 0 and again on day 6 immediately before mating, but the exposure of newly-emerged males to carbon dioxide for four to five minutes significantly inhibited feeding at two hours after treatment; the activity was normal 24 hours after treatment.

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