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Effects of Carbon Dioxide Anaesthetic on *Glossina*

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Abstract

Exposure of 2 day old virgin females of *Glossina fuscipes fuscipes, G. pallidipes, G. brevipalpis,* and *G. morsitans centralis* to carbon dioxide anaesthesia for 15 sec has the effect of suppressing their subsequent insemination frequency. This insemination-inhibitory effect of the gas is more pronounced in *G. f. fuscipes* and *G. pallidipes* than in *G. brevipalpis* and *G. m. centralis.* In *G. f. fuscipes* the adverse effect on insemination persists, albeit to a lesser degree, at least up to 72 hr. Carbon dioxide anaesthesia also reduces the insemination capability of *G. f. fuscipes* males; this effect, however, is less marked than in females. Exposure of wild non-teneral *G. f. fuscipes, G. pallidipes, G. brevipalpis,* and 3 day old *G. m. centralis* to the gas for 30 min causes some mortality during anaesthesia, which increases with increasing exposure period. *G. m. centralis* is most tolerant to the lethal effect of the gas within the 10–90 min exposure periods. Wild females of *G. f. fuscipes* and *G. pallidipes* seem to be more sensitive to carbon dioxide in this respect than males. In view of these adverse effects produced by carbon dioxide anaesthesia, its use on tsetse is not to be recommended.

Introduction

Of the various immobilizing agents, carbon dioxide has been by far the most widely used anaesthetic for ease in the handling of insects. However, an increasing number of investigators have found that this anaesthetic produces undesirable side effects in many insects (McCrady & Sulerud, 1964; Henneberry & Kishaba, 1967; Crystal, 1967; Hooper, 1970; White et al., 1970; Perron et al., 1972). In a study on functions of the male accessory glands of *G. f. fuscipes* Machado, preliminary experiments revealed that carbon dioxide anaesthesia was unsatisfactory as it resulted in reduced insemination frequency (Moloo, 1972). The present paper is concerned with further work on the effects of this anaesthetic on *G. f. fuscipes, G. pallidipes* Aust., *G. brevipalpis* Newst. and *G. m. centralis* Machado.

Materials and Methods

G. f. fuscipes, G. pallidipes and *G. brevipalpis* were caught from the South Busoga fly-belt of Uganda. The non-teneral wild flies or their offspring were used in the present study. *G. m. centralis* puparia were collected near Singida in Tanzania, and dispatched to EATRO, Uganda, in expanded polystyrene boxes (Kernaghan & Nash, 1964). Flies and puparia were kept in an insectarium

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maintained at 25 °C, 80 % r.h., and under a daily photoperiod of 12 hr subdued natural light. Flies were fed daily on live oxen. Those oxen which were used to feed wild flies were examined regularly for trypanosomes by the haematocrit centrifugation technique of Woo (1970), and when diagnosed positive were eliminated and treated. Emerging flies (0–24 hr old) were separated daily by sex to ensure virginity. Unless otherwise stated, 3 day old females were mated singly with 10 day old males, and the pairs left together for 24 hr.

Flies were placed either singly or in groups in a specially constructed glass chamber connected to a cylinder of commercial pressurized carbon dioxide. To ensure immediate exposure of flies to carbon dioxide, the gas was first released through the bottom 1-cm diameter inlet opening of the 30 × 30 × 10-cm chamber for 30 sec with the same size outlet opening in the top lid left open. At the end of this period, the experimental fly/flies in a Geigy cage were rapidly placed in the chamber and, with the top lid closed, a steady flow of the gas was continued for the required period at room temperature. The methods specific to particular experiments are described under appropriate sections.

Experiments and results

Insemination frequency

Two day old virgin females of *G. f. fuscipes, G. pallidipes, G. brevipalpis* and *G. m. centralis* were exposed singly to carbon dioxide for 15 sec. Similar females of each species served as untreated controls. The anaesthetized flies of all four species recovered within 1 min. Twenty-four hours subsequent to the treatment all the flies were mated with untreated males and then the females were dissected, and their spermathecae were examined under a phase-contrast microscope to determine insemination. Although pairing of the sexes occurred, insemination frequency of the treated flies was lower compared with their respective controls (Table I). The degree of insemination inhibitory effect, however, varied among the four species; insemination rates among treated insects of 8.9 %, 6.6 %, 41.4 % and 79.9 % of control values being recorded for *G. f. fuscipes, G. pallidipes, G. brevipalpis* and *G. m. centralis,* respectively.

Table I. Insemination frequency in four species of virgin female *Glossina* subjected to carbon dioxide anaesthesia and mated 24 hr later with normal males

Species	Treatment	Number of flies	Insemination frequency %
G. f. fuscipes	Anaesthetized	90	7.8
	Control	79	87.3
G. pallidipes	Anaesthetized	36	5.6
	Control	34	85.3
G. brevipalpis	Anaesthetized	102	26.5
	Control	97	64.0
G. m. centralis	Anaesthetized	65	74.1
	Control	70	92.7

Table II. Duration of insemination inhibitory effect of carbon dioxide anaesthesia upon virgin female *G. f. fuscipes* mated with normal males

Treatment	Interval after anaesthesia when mated h	Number of flies	Insemination frequency %
Anaesthetized	1	57	5.3
Control		18	88.9
Anaesthetized	24	25	4.0
Control		20	90.0
Anaesthetized	48	63	6.3
Control		40	80.0
Anaesthetized	72	39	28.2
Control		14	92.9

To investigate the duration of this effect in *G. f. fuscipes,* 2 day old virgin females were exposed to carbon dioxide for 15 sec, and after varying periods were mated with 10 day old untreated virgin males. Table II shows that the inhibitory effect of this anaesthetic on insemination rate was approximately the same up to 48 hr after exposure, and the effect persisted, albeit to a relatively lesser extent, at least for 72 hr.

Since *G. f. fuscipes* was originally selected for the study of functions of the male accessory glands, and since in the females of this species the adverse effect of carbon dioxide was markedly high, the effect of this anaesthetic on the insemination capability of the males was also investigated. Eighty 9 day old virgin males were individually exposed to the gas for 15 sec, and 24 hr later were mated with 3 day old untreated virgin females. Forty contemporary males served as untreated controls. The insemination frequency of the experimental flies was 52.5 % as against 85.0 % for the untreated controls.

Toxicity

Having established that carbon dioxide anaesthesia produces an insemination-inhibitory effect, an attempt was made to determine if this immobilizing agent is toxic to tsetse. Wild non-teneral males and females of *G. f. fuscipes, G. pallidipes, G. brevipalpis,* and 3 day old *G. m. centralis* were exposed to the gas for varying period from 10 to 300 min. Those flies which died during the exposure were scored; those which came to activity following the exposure were not scored even though some of these survived for only a short period.

The mortality of all four tsetse species increased with increasing exposure period (Table III). Also, the recovery period of survivors increased with increasing exposure to carbon dioxide. In all four species bloating of the abdomen with gas occurred in most of the flies during exposure particularly for exposures longer than 10 min. This bloating subsided shortly after flies were removed from the influence of the anaesthetic. The majority of flies which recovered after exposure of 120 min and longer seemed lethargic and either died soon afterwards

Table III. Mortality rate of four species of *Glossina* subjected to carbon dioxide anaesthesia for varying periods

Exposure period min.	*G. f. fuscipes*				*G. pallidipes*				*G. brevipalpis*				*G. m. centralis*			
	males		females		males		females		males		females		males		females	
	N	M	N	M	N	M	N	M	N	M	N	M	N	M	N	M
10	25	0	36	0	23	0	34	0	20	0	32	0	40	0	24	0
30	25	0	30	0	31	0	60	10.0	41	7.3	36	0	35	0	24	8.3
60	49	26.5	55	20.0	62	17.7	31	32.3	35	37.1	28	0	30	3.3	39	0
90	52	9.6	32	68.7	81	6.2	55	72.7	60	25.0	36	27.8	16	0	23	0
120	72	44.4	46	76.1	28	71.4	43	72.1	31	51.6	23	26.1	16	50.0	36	13.9
150	35	40.0	40	67.5	46	87.0	65	90.8	45	26.7	31	64.5	12	33.3	38	47.4
180	33	81.8	51	90.2	20	80.0	86	97.7	32	87.5	30	93.3	14	42.9	76	77.6
210	64	93.7	71	97.2	45	95.6	42	95.2	30	100	46	87.0	8	100	80	95.0
240	20	100	39	100	24	100	66	100	45	97.8	28	100	13	100	45	88.9
300	42	100	43	100	27	100	38	100	46	100	26	100	13	100	40	100

N = number exposed M = % mortality

or were found dead on the following day. The toxic effect of carbon dioxide anaesthesia was in general greater in females than in males of *G. f. fuscipes* and *G. pallidipes*. In the other two species, however, no such trend was detected. Table III also shows that for exposure periods up to 90 min *G. m. centralis* were less sensitive to the toxic effect of the gas compared with the other three tsetse species; longer exposure periods induced high mortality in all four species.

Longevity and reproductive performance

Since en exposure period of 15 sec to carbon dioxide has been used at the EATRO laboratory to immobilize wild as well as laboratory reared tsetse for

Table IV. Effects of carbon dioxide anaesthesia on the reproductive performance of wild females of *G. f. fuscipes, G. pallidipes* and *G. brevipalpis*

Species	Treatment	No. of flies	Mean survival after test (days)	No. of abortions	No. of normal pupae	Normal pupae/female	Pupae	
							Mean weight (mg)	*t*
G. f. fuscipes	Anaesthetized	229	6.6	3	28	0.12	26.8±1.2	0.086
	Control	208	7.8	1	23	0.11	26.2±1.1	
G. pallidipes	Anaesthetized	142	11.1	9	33	0.23	28.9±1.5	0.513
	Control	162	10.1	1	56	0.35	31.9±1.0	
G. brevipalpis	Anaesthetized	60	24.7	5	97	1.62	63.2±1.2	1.199
	Control	57	27.5	6	108	1.89	66.5±1.2	

Table V. Effects of carbon dioxide anaesthesia on the longevity and reproductive performance of *G. f. fuscipes*

Treatment	Males		Females				
	No. of flies	Mean longevity (days)	No. of flies	Mean longevity (days)	Pupae		
					No. produced	Mean weight (mg)	*t*
Anaesthetized	20	99.7	20	93.0	38	24.8±0.9	0.666
Control	20	118.3	20	87.3	38	25.4±1.3	

experimental manipulation, the effects of this technique on the longevity and reproductive performance of *Glossina* were investigated as follows:

(i) Wild females of *G. f. fuscipes, G. pallidipes* and *G. brevipalpis* were each divided in two groups. One group of each species was anaesthetized in batches of up to 25 flies while the other group served as control. Results (Table IV) showed that exposure of these flies to carbon dioxide anaesthetic for 15 sec produced no demonstrable effect on longevity or on reproductive performance. Nevertheless, the flies used in this experiment were wild and hence differed in their chronological as well as physiological age. Thus it is not possible to state with certainty that the anaesthesia had no effect.

(ii) Forty 4 day old males of *G. f. fuscipes* were divided into two equal groups; the flies of one group were anaesthetized individually while those of the other served as control. In another experiment, 4 day old mated females were used and the experiment was conducted in the same way. Again, the results (Table V) showed that the anaesthetic produced no effect on longevity or on reproductive performance.

Discussion

It has been demonstrated that short exposure of the cabbage looper moth, *Trichoplusia ni* (Hubner) to carbon dioxide reduces mating for 24 hr (HENNEBERRY & KISHABA, 1967). The present study shows that exposure of 2 day old virgin females of *G. f. fuscipes, G. pallidipes, G. brevipalpis* and *G. m. centralis* to carbon dioxide for as short a period as 15 sec has the effect of suppressing their subsequent insemination frequency. Whether this adverse effect on insemination is carbon dioxide specific or due to anoxia cannot be stated on the basis of the present experiments. The insemination-inhibitory effect of carbon dioxide anaesthesia is more pronounced in *G. f. fuscipes* and *G. pallidipes* than in *G. brevipalpis* and *G. m. centralis,* and suggests that there are interspecific differences in the sensitivity of tsetse to this gas. In *G. f. fuscipes* females the inhibition persists, albeit to a lesser degree, for at least 72 hr. However, since insemination rate increases as the period between exposure and mating is increased, it would seem that the adverse effect of carbon dioxide anaesthesia is not permanent. Carbon dioxide as an anaesthetic also reduces the insemination capability of *G. f. fuscipes* males, although to a lesser extent compared with its effect on

females. It is noteworthy that exposure of *G. m. morsitans* Westwood (*G. m. orientalis* Vanderplank) males to carbon dioxide for 4–5 min does not affect their insemination capability (BIRKENMEYER & DAME, 1970), again indicating interspecific differences in the sensitity of tsetse to this anaesthetic.

The present study also demonstrates a toxic effect of carbon dioxide anaesthesia. Exposure of wild non-teneral *G. f. fuscipes, G. pallidipes, G. brevipalpis,* and 3 day old *G. m. centralis* to the gas for 30 min causes some mortality which increases with increasing exposure period. Although all flies recovered some of these died not long after recovery following 10 min exposure, while for 150 min exposure and longer most flies which recovered died during the following 24 hr. The relatively greater tolerance to the toxic effects of carbon dioxide in the 10–90 min exposure periods of *G. m. centralis* compared with the other three species could be attributed to the following factors, operating either singly or in combination: (i) the *G. m. centralis* adults used were younger than the wild flies of the other three species, in which case greater sensitivity to carbon dioxide with increasing age must be postulated; (ii) the *G. m. centralis* adults emerged from pupae under laboratory conditions whereas wild flies were handled many times during the period between catching and setting up the experiment; hence the latter were possibly weaker; (iii) *G. m. centralis* is inherently more tolerant to carbon dioxide anaesthesia and/or anoxia.

BIRKENMEYER & DAME (1970) found that exposure of newly emerged (0–24 hr old) *G. m. morsitans* to carbon dioxide for 30–240 min does not affect their longevity. These authors reported that young flies (0–24 hr old) exposed for 30 min to this gas had increased survival, whereas exposure of older flies (2 day old) resulted in the reduction of survival of the males and not the females. However, in our experiments exposure of 3 day old *G. m. centralis* for the same period resulted in some mortality of the females alone. In the case of wild *G. f. fuscipes* and *G. pallidipes,* females seemed more sensitive to carbon dioxide anaesthesia within the 10–90 min exposure periods than males; no such trend between the sexes was observed in the other two tsetse species. Toxic effects of carbon dioxide have been described for other insects. For example, L'HÉRITIER (1948, 1958) found that certain *Drosophila* strains were unable to recover after a 30 sec exposure to carbon dioxide, and showed that such sensitivity was due to sigma virus. Virus-like particles have been observed in tsetse tissues (JENNI, 1973; JENNI & STEIGER, 1977), but whether such particles are responsible for the sensitivity of their hosts to carbon dioxide is unknown. An enhanced mortality due to carbon dioxide anaesthesia was reported in the Mediterranean fruit fly, *Ceratitis capitata* (Wied) (SHERMAN, 1953), and also in the Mexican fruit fly, *Anastrepha ludens* (Loew) (LOPEZ & BALOCK, 1970). PERRON et al. (1972) found that 10–15 min exposure to carbon dioxide resulted in high mortality of the 0–3 hr old *Drosophila melanogaster* Meig., but no such toxic effect was observed in flies older than 3 hr. Also, 0–3 hr old females were more sensitive to the gas than males.

Although the four species of *Glossina* studied show different reactions to carbon dioxide anaesthesia insofar as the degree of sensitivity is concerned, all four species are adversely affected by the gas. Hence the use of this anaesthetic on tsetse is not to be recommended.

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