# CARBON DIOXIDE ANESTHESIA IN PHLEBOTOMINE SAND FLIES (DIPTERA: PSYCHODIDAE): $CO_2$ EFFECT UPON TWO LABORATORY COLONIES

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ABSTRACT. Gravid females of 2 sand fly species, *Phlebotomus papatasi* and *P. perniciosus*, were exposed to carbon dioxide anesthesia for 5, 10 and 20 minutes. Recovery time, mortality at 0 min and 24 h, percentage of females laying eggs, time to oviposition, and egg productivity for each exposure time were registered. Survival, fecundity and oviposition time in the 2 species were not adversely affected by the short period of $CO_2$ anesthesia routinely used in the sand fly colony maintenance.

Phlebotomine sand flies (Diptera; Psychodidae) are vectors of *Leishmania* parasites, a variety of arboviruses and *Bartonella bacilliformis*. They are fragile, tiny insects and, though sometimes difficult to breed under laboratory conditions, it is now generally recognized that some species can be colonized in large numbers with relative ease (Killick-Kendrick et al. 1991, Lawyer et al. 1991). In our laboratory, two sand fly species, *Phlebotomus papatasi* and *P. perniciosus*, are bred in sufficient numbers to provide excess flies for experimental work.

The laboratory handling of adult sand flies includes: 1) isolation of engorged females individually or in pools for colony maintenance and experimental studies; 2) sexing, and 3) counting for colony productivity. Usually, the handling is time consuming and a major cause of death. In mass rearing sand flies, Lawyer et al. (1991) have developed an useful aspirator (vacuum-type) to transfer engorged flies. Some experimental procedures, i.e., parenteral injection of phleboviruses (Ciufolini et al. 1985) need immobilized flies. Among several methods reported in the literature, carbon dioxide is frequently used to anesthetize insects during sorting because it is safe and quick acting. For anesthetizing adult sand flies in colony maintenance and experimental studies we routinely use a simple $CO_2$ apparatus, previously described by Miceli et al. (1990).

Numerous papers have been published on the effects of $CO_2$ on various biological, physiological, and ethological characteristics of insects (Nicolas and Sillans 1989). Since information on $CO_2$ effects are gathered in different ways according to the authors' scope of study, there are difficulties in comparing results owing to differences in the insect populations tested and methods used. This study was undertaken to evaluate the effect of $CO_2$ anesthesia on *P. papatasi* and *P. perniciosus*. Parameters measured were recovery time, mortality rate and egg productivity of engorged females of the 2 species exposed to $CO_2$ anesthesia.

Sand flies used were from laboratory colonies of *P. papatasi* and *P. perniciosus* maintained since 1981. Groups of 5–6 day old females, bloodfed 24 h before, were exposed for 5, 10 and 20 min to a slow stream (750 ml/min) of $CO_2$ by using the apparatus described by Miceli et al. (1990). After exposure, females were tubed individually in glass vials (Maroli et al. 1987). Three replicates were carried out. Recovery times and number of dead flies were registered. The criterion used to estimate recovery was the ability of tested flies to stand and move about. Mortality rate at 0 min and 24 h, percentage of females laying eggs, oviposition time and number of eggs were calculated for each group of flies. Unexposed gravid females of both species from the same generations as those assayed for $CO_2$ were used as a control for oviposition time and egg productivity. Carbon dioxide exposure trials were carried out at room temperature (22–23°C) and 60–70% RH.

Table 1 shows recovery times and mortality rates of engorged *P. papatasi* and *P. perniciosus* females after different exposure times to $CO_2$ anesthesia. In general, the mean recovery times within 1 h in both species were correlated to the length of exposure. In *P. papatasi*, the percentages of females recovering within 60 min were always higher than 90%. The mean recovery times were between 9.6 and 25.7 min observed after 10 and 20 min of exposure, respectively. Only 5.6% of *P. papatasi* females exposed for 20 min did not recover by 1 h after, showing a mean recovery time of 106 ± 23 min. The mortality rates observed in *P. papatasi* at 0 min (range 1.4–4.7%) and 24 h (range 12.7–16.3%) showed no correlation with the length of time exposed to $CO_2$. In *P. perniciosus*, the percentages of females recovering before 60 min were between 100 and 69.8%, observed at 5 and 20 min of exposure, respectively. The mean recovery times

Table 1. Effects of $CO_2$ anesthesia on engorged females of *Phlebotomus papatasi* and *P. perniciosus*; recovery time and mortality rate

| Time of exposure (min) | Flies tested | Recovery <60 min | | Recovery > 60 min | | % mortality | |
|---|---|---|---|---|---|---|---|
| | | % | Mean time | % | Mean time | Starting mortality | 24 h |
| *P. papatasi* | | | | | | | |
| 5 | 43 | 95.3 | 12.1 ± 0.7 | 0 | 0 | 4.7 | 16.3 |
| 10 | 74 | 98.6 | 9.6 ± 0.3 | 0 | 0 | 1.4 | 13.5 |
| 20 | 54 | 90.7 | 25.7 ± 1.8 | 5.6 | 106 ± 23.0 | 3.7 | 12.7 |
| *P. perniciosus* | | | | | | | |
| 5 | 35 | 100.0 | 7.6 ± 0.3 | 0 | 0 | 0 | 11.4 |
| 10 | 41 | 90.3 | 22.3 ± 1.5 | 7.3 | 76 ± 4.9 | 2.4 | 7.3 |
| 20 | 43 | 69.8 | 32.6 ± 2.7 | 20.9 | 81 ± 6.9 | 9.3 | 7.8 |

Table 2. Effects of $CO_2$ anesthesia on engorged females of *Phlebotomus papatasi* and *P. perniciosus*; fecundity, mean oviposition time and egg producitivity

| Time of exposure (min) | Flies tested | % of females laying eggs | Mean oviposition time (days) | Mean eggs per female |
|---|---|---|---|---|
| *P. papatasi* | | | | |
| 5 | 34 | 91.2 | 5.7 ± 0.3 | 27.0 ± 2.2 |
| 10 | 63 | 66.7 | 6.4 ± 0.3 | 32.6 ± 2.5 |
| 20 | 45 | 73.3 | 5.5 ± 0.2 | 28.8 ± 2.5 |
| Unexposed | 27 | 88.9 | 5.8 ± 0.2 | 29.8 ± 2.3 |
| *P. perniciosus* | | | | |
| 5 | 31 | 80.6 | 5.8 ± 0.4 | 46.8 ± 2.4 |
| 10 | 37 | 81.1 | 5.1 ± 0.2 | 37.9 ± 2.2 |
| 20 | 36 | 86.1 | 5.3 ± 0.3 | 32.6 ± 1.8 |
| Unexposed | 50 | 92.0 | 5.6 ± 0.1 | 38.9 ± 1.7 |

which correlated well with the exposure times of 5, 10 and 20 min, were 7.6, 22.3 and 32.6 min, respectively. Some percentages of *P. perniciosus* (7.3% in 10 min of exposure and 20.9% in 20 min of exposure) showed a delayed-recovery of more than 1 h; on average 76 and 81 min, respectively. The mortality rates were similar to those observed in *P. papatasi*, showing no correlation with the exposure period.

The effect of $CO_2$ anesthesia on the fecundity, mean oviposition time and egg productivity of the 2 species are reported in Table 2. In general, $CO_2$ exposure did not have significant effect on the percentage of females laying eggs, their mean oviposition time and mean number of eggs laid per female in both species compared with the data obtained from control (unexposed) flies. All data observed are in the range of those of the controls. It seems that in *P. perniciosus*, the shortest period of $CO_2$ exposure could enhance the mean number of eggs/female; 46.8 ± 2.2, against 38.9 ± 1.7 of the controls.

Considering that in routine handling of colonies it is unlikely that adult sand flies are exposed to $CO_2$ for than 5 min, these findings suggest that $CO_2$ anesthesia is safe for gravid females. The mortality rate observed at 0 min and 24 h is the same as that usually observed in bloodfed females under laboratory conditions. Anesthesia with $CO_2$ allows one to collect engorged and/or infected females from laboratory colonies without consuming time. Miceli et al. (1990) reported that tubing engorged females individually from a laboratory breeding cage needs 20 and 46 secs per fly in anesthetized and nonanesthized flies, respectively. In conclusion, it seems that survival, fecundity and oviposition period in the 2 sand fly species studied are not adversely affected by the low exposure time to $CO_2$.

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