Response of *Liposcelis bostrychophila* and *L. entomophila* (Psocoptera) to carbon dioxide

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Abstract

Mixed-age samples of *L. bostrychophila* and *L. entomophila* were exposed to various concentrations of CO_2 (10–90%). *L. bostrychophila* was found to be the more tolerant of the two species. A linear correlation is indicated between the LT_{100}s and CO_2 concentrations for both species. Eggs are the most tolerant life stage for both species.

Mortality of 1–3 week old female liposcelids was determined at fixed CO_2 concentrations. *L. bostrychophila* was the more tolerant species when either 45 or 60% CO_2 was used. Increases in exposure periods resulted in corresponding increases in mortality in both species. Increasing the CO_2 concentration from 45 to 60% did not produce a significant change in response of *L. entomophila*. However, for *L. bostrychophila*, at 60% CO_2, an unexpected increase in exposure time was required to achieve the same level of kill. Currently recommended dosages of CO_2 are adequate for controlling both these species in well-sealed enclosures.

Introduction

Studies on the effects of fumigants (Pinniger 1985; Kalinovic 1984; S.H. Ho and R.G. Winks, unpublished data) and controlled atmospheres (Pinniger 1985; Leong 1986; Bell et al. 1990) on *Liposcelis* spp. have been limited. Leong (1986) investigated the effects of carbon dioxide (CO_2) on the mortality of *L. entomophila* at 28°C and 77% r.h. He noted that 30% CO_2 effected a 100% kill of *L. entomophila* female adults when exposed for 24 hours or more. However, at lower concentrations, mortality was significantly higher in samples exposed for longer periods. Bell et al. (1990) in his studies on the effects of CO_2 on *L. bostrychophila* noted that, at 40% CO_2, the time required to bring about control was greater than 8 and 12 days at 10 and 15°C, respectively. When a higher concentration of CO_2 (80–100%) was applied, the required time for control was 10 and greater than 7 days for the respective temperatures. These findings demonstrate the potentiating effect of increasing temperatures on CO_2 toxicity in *L. bostrychophila*. In the review paper by Annis (1987), the absence of information on the response of stored-product liposcelids to controlled atmospheres was obvious. Since then, apart from the two studies cited above, no other research has apparently been conducted. Therefore, the work reported here was undertaken to determine the response of *L. bostrychophila* and *L. entomophila* to CO_2.

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Materials and Methods

The CO_2 chambers were constructed from glass desiccators (180 mm diameter) (Fig. 1). All connections were grease-sealed to prevent leakage. Experiments were carried out at 30 ± 1°C and 75 ± 3% r.h. maintained by a 50% v/v glycerol solution (Johnson 1939; Braun and Braun 1958) in the lower compartment of the chamber.

Ninety–nine percent pure CO_2 from a cylinder (Singapore Oxygen Air Liquide Pte. Ltd (SOXAL)) was slowly released into the exposure chamber until the desired percentage of CO_2 in air was registered on a Riken Interferometer Model 18. The monitoring of the gas in the chamber was achieved with a closed-system design, so as not to dilute the gas during the process (Fig. 2). The interferometer was regularly calibrated against CO_2 standards (19.8%, 29.8%, 53.9%, 74.4% and 99.9%) obtained from SOXAL. Deviations were negligible over a period of 2 months. As gas from the cylinder tended to be cold and dry, the CO_2 was first passed through warm (30°C) 50% (v/v) glycerol solution before introduction into the chambers. This humidified the gas to 75% r.h. The oxygen concentration was estimated using Jay's (1984) data. A linear regression analysis of these data showed a good predicability of the oxygen concentration based on the CO_2 concentration in air ($r^2 = 0.994$).

The concentration of CO_2 was recorded both at the beginning and end of the experiment. For experiments studying the time-to-100% kill of the liposcelids, the concentrations were checked daily and replenished when necessary. Chambers with daily deviations in concentrations of greater than 3% when checked were not considered in the final analysis.

Time-to-100% kill

Mixed-age samples of *L. bostrychophila* and *L. entomophila* (> 150 individuals per cage) were exposed to various concentrations of CO_2 (10–90%). Fifty adults were isolated in each cage and set aside for 4 weeks to obtain the required mixed-age samples. By the end of this time, adults, nymphs and eggs of various ages were present. Five cages were placed in each exposure chamber.

The LT_{100} was determined by the method of inverse sampling (Finney 1971). At each fixed concentration, various exposure periods (at 24-hour intervals) were randomly selected. Upon termination of the treatment, cages were placed in incubators at 30 ± 1°C, 75 ± 3% r.h. and inspected weekly. Initial observations indicated that eclosion occurred within 2 weeks after treatment. A 2-week holding period was thus selected to include any delays in eclosion of the surviving eggs. Samples with surviving nymphs or adults were immediately discarded, while samples without surviving nymphs or adults were set aside for the respective holding periods before egg mortality was ascertained. The experiments were repeated with the exposure periods increased or decreased (by 24 hours) depending on the outcome of the preceding results.

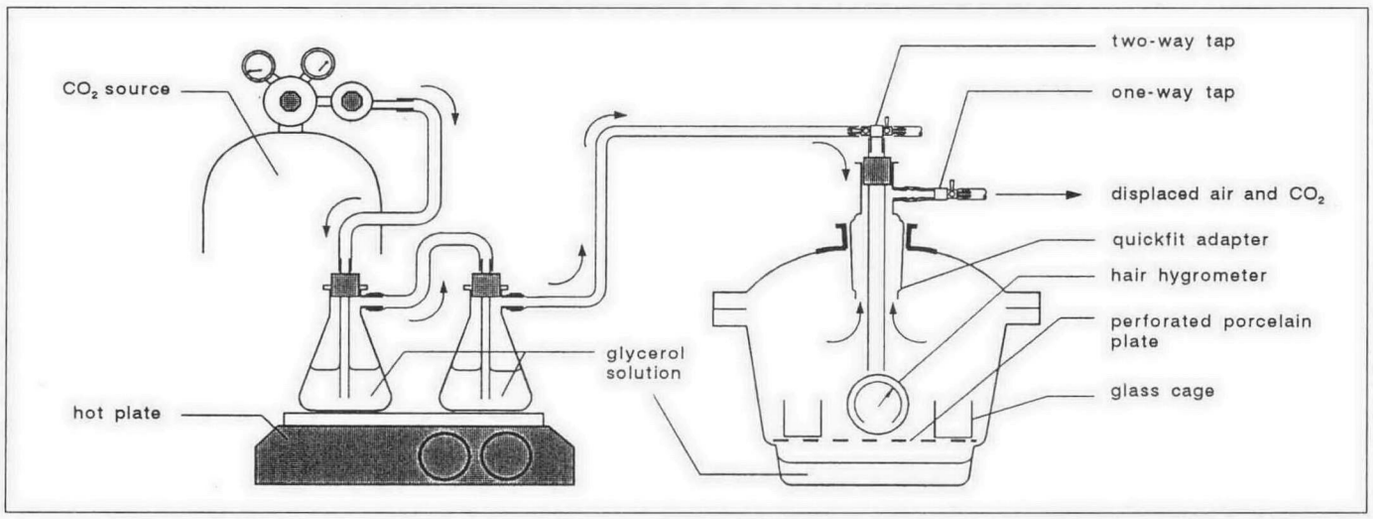

Fig. 1. Schematic diagram of the experimental set up. Pure CO_2 discharged from the cylinder is first conditioned before being channelled into the exposure chamber. The mixture of air and CO_2 displaced is then passed through soda lime before being released into the surroundings.

When the apparent LT_{100} for a particular concentration was obtained, the dosage was replicated 3 more times to confirm the time-to-100% kill. If survivors were noted in any one replicate, the exposure period was again increased by another 24 hours for the next treatment. Similarly, three replicates for confirmation were conducted. The results were analysed by linear correlation using the Maximum Likelihood Program (MLP).

End-point mortality determination

The mortalities of the two species exposed to 60% CO_2 were monitored until end-point mortality was attained or when control mortalities exceeded 20%. This was replicated at least five times. The corrected mortalities were plotted against time and the end-point mortality determined graphically.

Response of female liposcelids to fixed concentrations of CO_2

L. bostrychophila and *L. entomophila* were cultured as described by Leong and Ho (1990) and Leong (1993). Females1–3 weeks old were used in all experiments. Batches of at least 40 liposcelids were isolated into each cage and counted. Three cages were placed in each exposure chamber and this was replicated up to 12 times per dosage. The variation in replicates used was dependent on the availability of test specimens.

Mortality response of 1–3-week-old female liposcelids was determined at two fixed concentrations (45 and 60%) of CO_2 with increasing exposure periods from 2 to 6 hours at half-hour intervals. Controls consisting of a similar number of cages in the exposure chambers not dosed with CO_2 were set aside for 4 hours. Preliminary trials showed that mortalities in the controls did not vary amongst the various exposure periods. Replicates with high control mortalities and for which changes in CO_2 concentrations before and after the experiment exceeded 3% were disregarded in the final analysis.

Results and Discussion

Time-to-100% kill

It is obvious from the results (Fig. 3) that *L. bostrychophila* is the more tolerant of the two species to CO_2 treatment. A

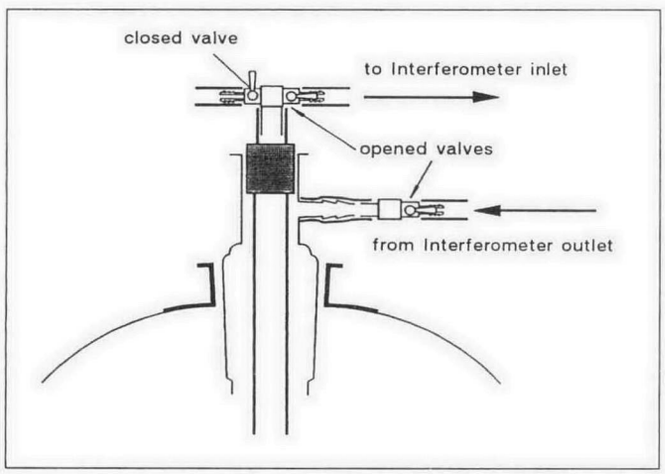

Fig. 2. Monitoring of CO_2 in the exposure chamber. The 'closed-system' adopted prevents dilution of the CO_2 concentration within the chamber.

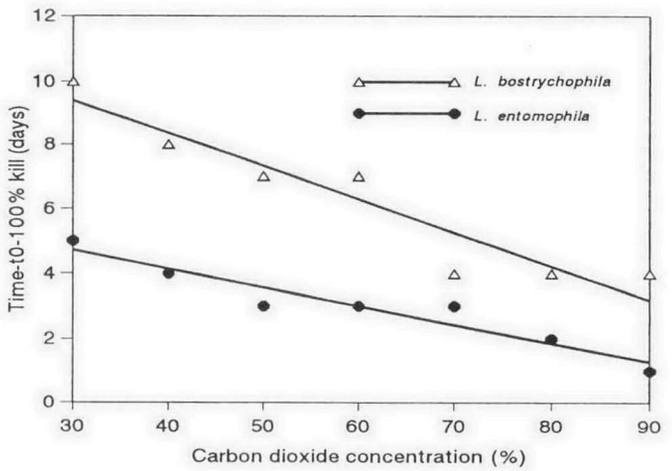

Fig. 3. Time-to-100% kill of mixed-age individuals of *Liposcelis* spp to various concentrations of CO_2 at 31 ± 1°C and 75 ± 3% r.h.

linear correlation is suggested between the LT_{100}s and CO_2 concentrations for *L. entomophila* and *L. bostrychophila*. Although no significant difference ($P > 0.05$) was noted between the slopes of the two plots, significant displacements ($P < 0.01$) were noted (Table 1). Generally, increasing concentrations decreased the time-to-100% kill of both species. The most tolerant life stage for both species is the eggs. Neither adults nor nymphs survived the treatments. This is not surprising as the 'inactive' stages (eggs or pupae if present) have been found to be the most tolerant life stage in many insects treated with CO_2, with the exception of a few larval stages of some stored product beetles (AliNiazee 1971; Bailey and Banks 1980; Jay 1984; Ho et al. 1987; Annis 1987). Literature on the lethal effects of CO_2 on insect eggs is limited. It is possible that the toxicity of high CO_2 concentrations to the eggs of insects may be due to interference with the normal metabolic growth process of the eggs or the anaesthetic action of the gas on the embryo's nervous system. Bell (1984) reported increased sensitivity to CO_2 in early eggs of pyralid moths; however, in the presence of oxygen, sensitivity decreased as embryogenesis proceeded. Furthermore, he reported that, in the presence of oxygen, development is delayed with no evidence of cessation, suggesting that death results from progressive CO_2 poisoning or the accumulation of toxic products otherwise removed via oxidative metabolism. Interestingly, during anoxia the development of eggs virtually ceases (Price and Bell 1981), and thus survival depends on the capacity of the embryo to accumulate glycolytic products and reduce its needs for active metabolism.

The observed differences in LT_{100}s of the two liposcelid species can at best be attributed to interspecific differences in tolerance to CO_2. The shorter incubation period of *L. entomophila* eggs (3–4 days shorter than *L. bostrychophila* eggs), and the different modes of reproduction (*L. bostrychophila* being parthenogenetic), may be responsible for the observed differences. From the available information (Press and Flaherty 1973; Bell 1984), it is clear that the relation of egg age and CO_2 toxicity is a complex one, with no one simple trend that can best describe this relationship.

End-Point mortality determination

Five and 8-day post-treatment holding periods were found to best reflect the end-point mortalities of *L. entomophila* and *L. bostrychophila*, respectively (Fig. 4). To avoid high control mortalities, post-treatment holding periods longer than those suggested above are not recommended, especially for *L. entomophila*. Although mortalities recorded after a 24 hour post-treatment period for both species provide good estimates of end-point mortalities, the monitoring of mortalities over several more days is recommended (limited by high natural mortalities) to accommodate variations expected in end-point mortalities for different dosage regimes. Winks (1984, 1986) noted the importance of response time (defined as the time that elapses between the administration of a dosage of a drug or poison and the expression of the response to that dose) when assessing mortalities for different dosages of phosphine. Hence, variations amongst the replicates recorded over the duration monitored could be attributed to population variations in not only tolerance to CO_2 but also response time to the treatment.

Response of females liposcelids to fixed concentrations of CO_2

L. bostrychophila is the more tolerant ($P < 0.05$) species when either 45 and 60% CO_2 was used (Fig. 5). Increases in exposure periods resulted in corresponding increases in the mortality in both species. Increasing the concentration of CO_2 from 45 to 60% did not produce a corresponding increase in mortality for *L. entomophila*. For *L. bostrychophila*, at 60% CO_2 an unexpected increase in exposure time was required to achieve the same level of kill as 45% CO_2. More data are needed from further work on the response of these liposcelids to a range of CO_2 concentrations at fixed exposure periods.

In comparison with other insects reviewed by Annis (1987) in terms of LT_{100}s for various concentrations of CO_2, it is apparent that female *L. entomophila* and *L. bostrychophila* rank amongst the more susceptible. The effect of CO_2 on the

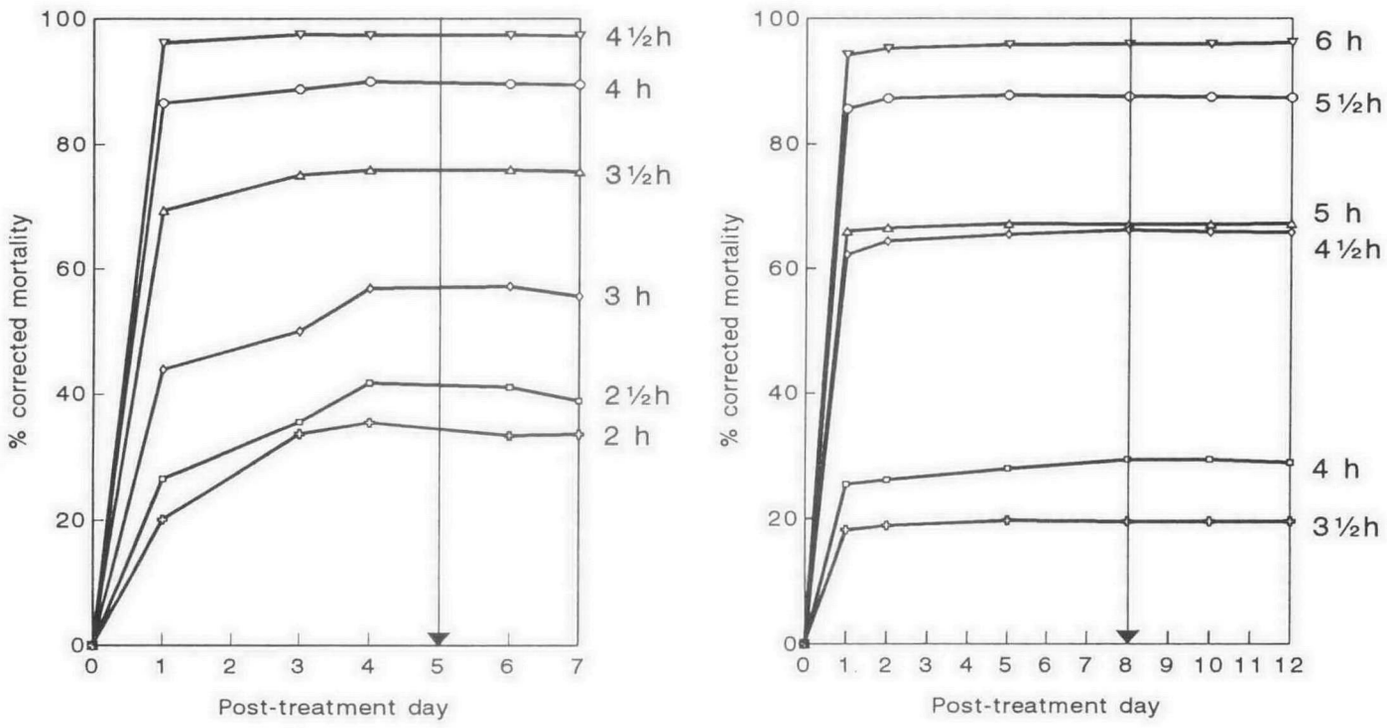

Fig. 4. End-point mortality determination in *L. entomophila* (left) and *L. bostrychophila* (right) at 60% CO_2, 31 ± 1°C and 75 ± 3% r.h. The arrows indicate the time which reflect end-point mortality.

Table 1. Linear regression of LT_{100}s against CO_2 concentration for mixed-age samples of *L. entomophila* and *L. bostrychophila* (mean ± s.e.).

	Species			
	L. entomophila	*L. bostrychophila*	d.f.	F-ratio[a]
Intercept	7.00 ± 0.61	12.50 ± 0.98	5	73.89**
Slope	-0.064 ± 0.010	-0.104 ± 0.016	5	4.618 ns
R-value	0.938	0.934	—	—

[a] Derived from Analysis of Parallelism table from the MLP output where the F-ratios for the intercept and slope were calculated from: $MS_{Displacement}/MS_{Within\ group}$ and $MS_{Parallelism}/MS_{Within\ goup}$, respectively.

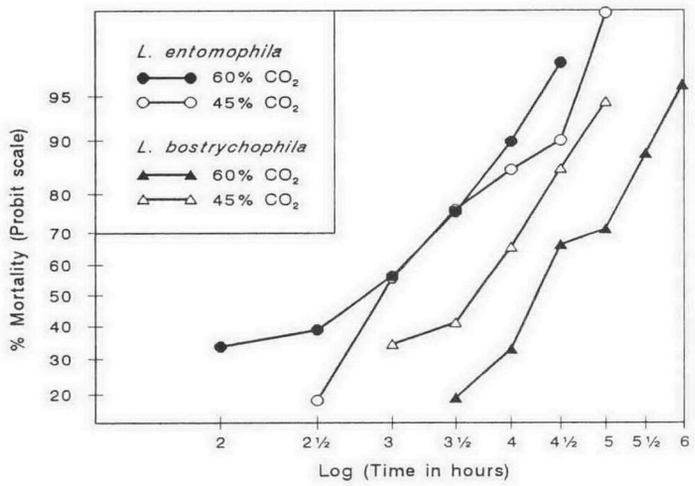

Fig. 5. Mean plots of the mortality of *L. entomophila* and *L. bostrychophila* (1–3 week old females) treated with 45% and 60% CO_2 for various exposure periods, showing the relatively linear association between the two parameters.

stimulation of prolonged spiracular opening (Hoyle 1960; Miller 1974), and thus water loss, has been suggested to contribute to the overall toxicity of CO_2 in the liposcelids (Leong 1986). Moreover, the CO_2-permeability of the integument of these soft-skinned insects (Wigglesworth 1972) coupled with their heavy dependence on the active physiological absorption of water from the atmosphere (Devine 1978) would also play a significant role in the susceptibility of liposcelids to hypercarbic atmospheres. However, the eggs of these liposcelids are noted to be amongst the most tolerant when compared with other stored-product insects' eggs (Annis 1987).

Conclusion

In conclusion, this work provides the baseline data for the response of *L. bostrychophila* and *L. entomophila* to CO_2. Although the eggs are more tolerant than many of the species recorded by Annis (1987), the latter's suggested CO_2 dosage regimes are adequate for controlling the liposcelids in well-sealed enclosures.

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