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EFFECT OF CARBON DIOXIDE ON *Prostephanus truncatus* (HORN)

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ABSTRACT

Susceptibility of exposure of eggs, larvae, pupae, and adults of *Prostephanus truncatus* (Horn) to concentrations of 40, 60, and 80% CO_2 in air at 30±2°C and 60±3% r.h. was investigated. For eggs and adults, differences in susceptibility were recorded between 40 and 60% CO_2, but not between 60% and 80%. A 95% mortality was obtained at 60% CO_2 after 2 days exposure of eggs, and 8 days exposure for first instar larvae, while 10 days exposure were required to produce the same mortality level of second and third instar larvae. The most tolerant stages were second and third instar larvae whereas eggs and adults were more susceptible.

INTRODUCTION

From the early 1970s the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera Bostrichidae) has exhibited a remarkable increase in distribution from Central America to other geographical areas, causing heavy damage, mainly to maize in the field and in storage. In particular *P. truncatus* has established itself in countries where maize is stored for long periods on the cob, and has rapidly reached very high population densities, causing a real threat to maize cultivation (Hodges, 1986). In some subtropical areas (Israel, Irak, and southern USA), although found on imported maize, the insect has not become established permanently, while mainly in Africa, it has been recorded frequently over the past decade and is now the most serious pest of this cereal grain (Golob, 1988; Hodges, 1986; Laborius, 1987).

The spread of this insect has been favoured greatly by the paucity of control methods at the farmer level where to date only simple smoking and drying procedures of maize cobs are carried out in infested areas (Golob and Hodges, 1982). Moreover, the fact that it is a xylophagous species has hampered control, even more so as warehouse structures in infested countries are generally wooden.

According to Schulz and Laborius (1987) chemical treatments are at present the only effective method of controlling this pest. According to Golob (1984), phosphine or methyl bromide effectively controlled the insect on

maize and cassava. Also pyrethroids and their admixture with organophosphate insecticides were found useful for control (Berg and Biliwa, 1989a,b, 1990; Giles and Leon, 1975; Golob, 1988; Golob and Hanks, 1990; Makundi, 1991; Pierce and Schmidt, 1992). Harris (personal communication in Hodges, 1986) stated that in silos the susceptibility of the larger grain borer to phosphine was very similar to that of other insect species infesting cereal grains.

The use of controlled atmospheres could provide an efficient alternative to chemical control, but until now, relevant data on the susceptibility of *P. truncatus* has not been available. In this study the effect of different concentrations of CO_2 on different stages of the insect's development was studied to find exposure times required for control.

MATERIALS AND METHODS

The culture stock originated from Equatorial Africa. The effectiveness of CO_2 in the control of *P. truncatus* was examined in the laboratory. The insects were reared on maize in a thermostatically controlled incubator at 30±1°C and 60±5% r.h. Adults (5-10 days old) and pupae were separated by sieving heavily infested (dusty) material from different cultures as this species can also develop on compressed maize kernel dust finely chewed by adults (Bell and Watters, 1982). Eggs were also separated by sieving, and were exposed to CAs at 1-4 days old.

To obtain larval stages, the eggs were placed in containers (Ø 5 cm; h 10 cm) containing maize kernel dust finely chewed by adults, and after 3-4 days, 8-10 days, and 14-18 days, the material was sieved to separate out 1st, 2nd and 3rd stage larvae, respectively. Tests with prepupae were not carried out as the prepupa is regarded as a separate phase beginning when the last instar becomes immobile (Bell and Watters, 1982).

Insect exposure: The first stage of the experiments was carried out by putting the insects on a thin stratum of maize kernel dust, finely chewed by adults, in plastic cylindrical containers (Ø 1.5 cm; h 1 cm), placed inside a glass jar (Ø 8 cm; h 17 cm), equipped with an airtight metal alloy cap suitable for purging with CO_2. In order to obtain a relative humidity inside the jars suitable for insect development (60% ±3% r.h.), 50 g of wheat was placed in each jar. In fact it was found that the r.h. inside the jars after purging with dry gas (CO_2 plus air) decreased to 15-20% r.h., and then subsequently increased and stabilized after 60 min at the values indicated above. This verification was carried out on three jars similar to the ones used during the tests but equipped with humidity sensors that passed through the stopper to a previously calibrated Shaw hygrometer.

A flowmeter was adjusted to control the rate of introduction of the mixtures into the jars at 5 cm^3/min. Preliminary tests of gas purge were

carried out in containers similar to those used for the experiments using pure CO_2, to determine the time required for complete replacement of the air. The gas was introduced for 4 min into a partially open jar that was subsequently closed. The jars were sealed at positive pressure (0.1 bar) to permit verification of airtightness at the end of the experiment by the presence of a slight inflation of the cap. Dry gas purges were carried out at 25±1°C at three different concentrations of 40%, 60%, and 80% CO^2 in air .

Upon completion of the purge, the jars were put in a constant temperature chamber at 30±2°C and held until the end of the exposure period. Possible egg hatch was determined at the end of the exposure by incubating the eggs and counting egg hatch after 10 days. For larvae, pupae, and adults, the number of surviving insects was recorded at the end of the exposures and re-checked after 48 h to take into account the possibility of delayed death. Those insects that failed to move during mortality counts, even after subjection to vibration, light, and mild heat, were considered to be dead. For every experiment, 20 individuals were used, and each experiment was replicated 3 times. An untreated batch was used as control.

Insect exposure within food medium: During the second stage of the study, the efficacy of CO_2 in the control of *P. truncatus* was evaluated by simulating a silo infestation. The infested material (250 g of infested kernels and 30 g of maize dust finely chewed by adults) was obtained from mass-reared cultures and mixed to form homogeneous samples. The same jars were used as in the previous experiments. Each test was repeated 3 times, and accompanied each with an untreated control batch. The CO_2 was introduced using the same methods as in stage one of the experiments, employing those exposure periods that had resulted in deaths higher than 95% of the most resistant stage at different concentrations in the previous experiments. At the end of the treatments, the samples were incubated after surviving adults had been counted and removed. This was performed by weekly examination of the samples, until a maximum of 5 weeks, in order to permit the development of immature insects to the adult stage.

The treatments were evaluated on the basis of adult survival in comparison with the untreated control batches. Results were evaluated by analysis of variance using the Tukey test.

RESULTS

Eggs were very sensitive, with a 2 day exposure being sufficient, at all concentrations, to obtain a mortality higher than 95% (Table 1). For 1st instar larvae (Table 1), the 40% CO_2 concentration produced a mortality higher than 95% after 10 days, while at 60%, 8 days were needed. A 3-day treatment of 80% CO_2 was sufficient to obtain the same mortality. Second and third instar larvae (Table 1) were barely sensitive to the treatments. At

40% CO_2 in particular, 18 days were required to obtain above 95% mortality while at concentrations of 60% and 80% CO_2, 10 and 4 days were needed, respectively. Regarding pupae, the results were extremely heterogeneous, therefore further tests are needed. For adults a 10 day treatment was required at 40% CO_2 to obtain mortality higher than 95%, while 2 days sufficed for other concentrations (Table 1). The 40 and 60% CO_2 treatments yielded significantly different results for all stages; in the case of eggs and adults the same exposure periods at 60% and 80% CO_2 were required to obtain 95% mortality.

Table 1a: Average survival (±S.E.) rate of adults, eggs, and 1st instar larvae of *Prostephanus truncatus* (Horn) exposed to different concentrations of CO_2 in air at 30±2°C and 60±3% r.h.

	Exposure (days)	40% CO_2	60% CO_2	80% CO_2
ADULTS	0	20.00 a	20.00 a	20.00 a
	1	18.00±1.00 a	4.66±0.88 b	2.00±1.00 b
	2	14.66±0.33 b	0.33±0.33 c	0.00 b
	3	11.66±0.66 c	0.00 c	
	4	9.66±0.33 c		
	5	6.66±0.88 d		
	6	3.66±0.33 e		
	7	2.00±5.77 e,f		
	8	1.66±0.33 e,f		
	9	1.33±0.33 e,f		
	10	0.33±0.33 f		
	11	0.00 f		
EGGS	0	20.00 a	20.00 a	20.00 a
	1	3.66±1.45 b	1.00±0.33 b	0.00
	2	0.33±0.33 b,c	0.00 b	
	3	0.00 c		
1ST INSTAR LARVAE	0	20.00 a	20.00 a	20.00 a
	1	15.00±0.57 b	12.66±0.33 b	2.33±0.88 b
	2	14.66±0.33 b	10.00±0.57 c	1.66±0.33 b,c
	3	12.00±0.57 c,d	6.00±1.00 d	0.66±0.33 b,c
	4	12.33±0.33 c	5.66±0.66 d	0.33±0.33 b,c
	5	10.00±0.57 d,e	4.33±0.33 d	0.00 c
	6	8.00±0.57 e	3.66±0.33d,e	
	7	5.66±0.33 f	1.66±0.33e,f	
	8	4.00±0.57 f	0.66±0.33 f	
	9	1.33±0.33 g	0.00 f	
	10	0.00 g		

Values in a column followed by a different letter are significantly different at $p<0.05$.

Table 1b: Average survival (±S.E.) rate of 2nd and 3rd instar larvae of *Prostephanus truncatus* (Horn) exposed to different concentrations of CO_2 in air at 30±2°C and 60±3% r.h.

	Exposure (days)	40% CO_2	60% CO_2	80% CO_2
2ND and 3RD INSTAR LARVAE	0	20.00 a	20.00 a	20.00 a
	1	19.00±0.33 a,b	17.66±0.66 a,b	18.00±0.57 b
	2	17.33±0.66 a,b,c	15.33±0.88 b	5.33±0.33 c
	3	15.66±1.20 b, c	11.66±0.88 c	3.66±0.33 d
	4	14.66±0.88 c,d	10.00±1.00 c,d	0.33±0.33 e
	5	11.66±0.88 d,e	9.66±0.88 c,d	0.33±0.33 e
	6	10.33±0.66 e,f	7.33±0.33 d,e	0.00 e
	7	11.66±0.33 d,e	5.00±0.57 e,f	
	8	8.00±1.00 e,f,g	4.33±0.88 e,f	
	9	7.33±0.33 f,g,h	2.66±0.33 f,g	
	10	6.66±0.88 f,g,h	0.33±0.33 g	
	11	8.66±1.20 e,f,g	0.00 g	
	12	6.66±0.66 f,g,h		
	13	6.00±1.00 g,h,i		
	14	6.00±0.57 g,h,i		
	15	4.00±0.57 h,i,l		
	16	2.66±0.66 i,l,m		
	17	1.00±0.57 l,m		
	18	0.33±0.33 l,m		
	19	0.00 m		

Values in a column followed by a different letter are significantly different at $p<0.05$.

As to the effectiveness of CO_2 on material infested by *P. truncatus*, it was shown that 18 days were required at 40% CO_2 and 15-day treatment at 60% and 80% CO_2 to obtain 95% mortality of all stages, (see Table 2). After a 15 days treatment at the lowest CO_2 concentration, adults survived while at the other concentrations complete mortality was obtained after 10 days. For the immature stages, complete mortality was obtained after 15 days for exposure to 60% and 80% CO_2, and for exposure to 40% CO_2 individuals of the immature stages survived at 20 days) (Table 2).

CONCLUSIONS

The effectiveness of using CO_2 in *P. truncatus* control depends on the developmental stage and CO_2 concentrations employed. Eggs proved to be very sensitive, even at 40% CO_2, as compared with 2nd and 3rd instar larvae. Research on pupae must be continued as the wide range of results is

probably due to differences in susceptibility to CO_2 at the various pupal phases, particularly between newly formed pupae and those prior to adult emergence. The sensitivity of the prepupal stage is also worthy of consideration.

Table 2: Average survival (±S.E.) rate of adults and immature stages of *Prostephanus truncatus* (Horn) in 250 g infested kernels and 30 g finely ground maize dust exposed to different concentrations of CO_2 in air at 30±2°C and 60±3% r.h., and of untreated control batch.

ADULTS

Exposure (days)	40% CO_2	60% CO_2	80% CO_2
10 *	34.00±5.56 a	0.00	0.00
**	317.00±57.24 b	196.33±4.26	193.00±7.57
15 *	5.66±1.87 a		
**	242.00±7.02 b		
18 *	0.00		
**	250.33±2.19		
22 *	0.00		
**	215.33±12.78		

IMMATURE STAGES

Exposure (days)	40% CO_2	60% CO_2	80% CO_2
10 *	43.00±3.60 a	10.66±2.33 a	7.00±1.73 a
**	160.00±11.01 b	155.33±15.24 b	156.00±12.50 b
15 *	10.00±1.52 a	0.00	0.00
**	122.33±6.11 b	169.33	176.00±9.64
18 *	2.00±0.57 a		
**	190.00±14.29 b		
22 *	0.66±0.33 a		
**	185.33±11.92 b		

* Treated samples.

**Controls.

Values in a column followed by a different letter are significantly different at $p<0.05$.

The exposure periods required to obtain insect mortality at the different stages are prolonged because this species develops inside kernels or in finely chewed maize kernel dust. During the tests carried out on larvae and adults placed directly on maize kernel dust, the insects tended to penetrate the substrate and therefore they were relatively protected since the diffusion of CO_2 is obstructed by the presence of the maize kernel dust. It was already observed that immature stages of the species that develop inside the kernels are less sensitive to CO_2 (Annis, 1987; Lindgren and Vincent, 1970).

In principle, CO_2 can be used to control *P. truncatus* although long exposure periods are needed. The possibility of using this method entails a precise knowledge of the biology of the insect and the operative conditions, as the treatment periods necessary to obtain satisfactory results depend on CO_2 concentration, temperature, and humidity as well as the presence of finely chewed maize dust in the substrate (Annis, 1987; Jay, 1984; Navarro and Calderon, 1980). It also necessitates the storage of maize in well-sealed silos, a condition that is rarely met in the areas more frequently infested by the larger grain borer.

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