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Lethal effects of sulfuryl fluoride (SF) to developmental stages of two *Callosobruchus* species (Coleoptera: Chrysomelidae Bruchinae)

OFUYA, T.I.[1], REICHMUTH, C., KLEMENTZ, D., ADLER, C. and ADARKWAH, C.
Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Products Protection, Koenigin-Luise Strasse 19, 14195 Berlin, Germany.

ABSTRACT: The mortality and sub-lethal responses of developmental stages of *Callosobruchus maculatus* Fabricius and *C. subinnotatus* Pic. after fumigation with sulfuryl fluoride (SF) were observed under controlled conditions (25° and 30° C and 60-70% r.h.). Five SF concentrations (range, 3.1 – 20.3 g/m^3) and six exposure periods (range, 0-24 hours) were involved. Eggs, larvae, pupae and adults of the bruchids were fumigated in gastight dressel flasks and SF concentrations were determined with aid of a Fourier transform infrared spectroscope. The eggs of *C. maculatus* and *C. subinnotatus* were more tolerant to SF than the adults, larvae and pupae which were all killed by fumigation with 10.2 g/m^3 of SF at 30° C in 24 hours. Complete mortality of adults, larvae and pupae of the two bruchid species was achieved with 3.1 g/m^3 or more of SF at 25° C. Adults of bruchids were killed with 3.1 g/m^3 SF in 4 hours at 30° C. The developmental period (egg to adult) of fumigated bruchid eggs was significantly longer than for those that were not fumigated, by 2 to 4 days. Adult bruchids which survived direct sub-lethal exposure to fumigation with 3.1 g/m^3 SF laid significantly fewer eggs than those that were not exposed to fumigant. Adults that emerged from fumigated bruchid eggs laid as many eggs (P > 0.05) as those emerging from the eggs which were not fumigated.

Keywords: Sulfuryl fluoride; *Callosobruchus* species; control

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INTRODUCTION

Species in the genus *Callosobruchus* (Coleoptera: Chrysomelidae Bruchinae) are very serious pests of stored pulses in the tropics and subtropics (Ofuya, 2001). They infest and attack stored seeds leading to reduced weight, poor quality and loss of viability. *C. maculatus* Fabricius and *C. subinnotatus* Pic., that principally damage cowpeas and bambara groundnuts respectively, are perhaps the most prominent especially in the West African sub-region. For example, infestation of cowpeas in tropical Africa by *C. maculatus* alone can lead to seed losses of up to 30% within 6 months of storage with over 70% seed contamination, that makes the edible seeds unfit for human consumption (Ogunkoya and Ofuya, 2001).

Bruchid infestation of stored grain legumes is readily curtailed by the application of synthetic insecticide dusts such as pirimiphos-methyl and permethrin, and fumigation with methyl bromide

[1]Correspondence to: Ofuya, T.I., tomofuya@yahoo.com

and phosphine (Ofuya, 2001). However, these effective synthetic insecticides have some drawbacks such as development of resistance by insect pests and adverse effects on the environment. *C. maculatus* resistance to pirimiphos-methyl treatment has been reported in Nigeria (Odeyemi *et al*., 2006). Phosphine resistance has reached sufficient levels to cause control problems in developing countries where there has been widespread misuse of the fumigant (Bell *et al*., 2003; Yuchi *et al*., 2008). Methyl bromide has been identified as an ozone depleting chemical and its use is to be discontinued in all countries by 2015 under an international agreement known as the Montreal Protocol (Bell, 2000; Thoms *et al*., 2008). Prior to the 1980s, the fumigant sulfuryl fluoride (SF) has been used successfully for the control of termites in wood (Price, 1985). However, SF is now being registered for use for the control of stored products pest insects in many parts of the world (Thoms *et al*., 2008). SF is a biologically active inorganic chemical that is colourless, odourless, non-corrosive and non-flammable and essentially non-reactive in working airborne concentrations (Schneider *et al*., 2003). There has not been adequate research on the lethal effects of SF to bruchid pests of stored legumes. This paper reports mortality and sub-lethal responses of developmental stages of *C. maculatus* and *C. subinnotatus* after fumigation with SF.

MATERIALS AND METHODS

Eggs, larvae, pupae and adults of *C. maculatus* and *C. subinnotatus* used in this study were obtained from cultures maintained at Institute for Ecological Chemistry, Plant Analysis and Stored Products Protection, Berlin, Germany at 26° C and 65 – 70% relative humidity. Mung bean seeds were used as hosts in rearing the beetles and were also used for this experiment. The developmental stages of the two bruchids were treated at the following ages: adults (< 3 days), eggs (< 2 days), larvae (12-15 days) and pupae (0-3 days) following the methods of Mbata and Reichmuth (1996) and Ofuya and Reichmuth (2002). Dissected infested seeds were observed to confirm insect stages being treated with respect to larvae and pupae. Batches of 50 eggs on approximately 20 seeds, 50 larvae or pupae within the seeds or 20 adults, were placed separately in wire cages (8.0 cm long and 1.5 cm diameter) closed with rubber stoppers and exposed to SF in gastight dressel flasks. The moisture content of the air in the flasks were adjusted to about 65% by air recirculation through a saturated solution of $NaNO_2$ (Solomon, 1952) with the aid of a pump (Baltaci *et al*., 2009). The developmental stages were exposed to SF with the aid of A Fourier transform infrared spectroscope (FTIR) (GASMET) as clearly described by Baltaci *et al*. (2009). The target SF concentrations for adults were 3.0, 5.0 and 10.0 g/m^3 and those for eggs, larvae and pupae were 3.0, 5.0, 10.0, 15.0 and 20.0 g/m^3. However, the actual concentrations in the dressel flasks during fumigation as indicated on the computer are summarized in the results. The exposure period to the respective concentrations for all developmental stages was 24 hours, and at two different temperatures of 25° and 30° C. For each developmental stage and at each temperature, there was a control which was not exposed to SF but also humidified. The treatments for each developmental stage were replicated three times. The replication was simultaneous because a desired concentration was often difficult to achieve when repeated.

Emergence of adults from exposed eggs, larvae and pupae, and outright death of exposed adults were observed and recorded as described by

Mbata and Reichmuth (1996) and Ofuya and Reichmuth (2002). Mortality in the adults was checked 24 hours after the exposure period, at which time insects putatively anaesthesized by SF were expected to have recovered. For the eggs, larvae and pupae, the treated seeds were placed separately in dishes and incubated at 30° C and adult emergence was recorded after sufficient time for completion of development (Ofuya, 2001). Percent mortality in each stage was calculated and corrected using Abbott's (1925) procedure.

The mortality of freshly emerged adults when fumigated with SF at a concentration of 3.1 ± 0.08 g/m^3 and exposed over different periods of 0.5, 1, 2, 3, 4 and 5 hours at 30° C was also determined.

The sub-lethal effects of exposure of the eggs and adults of the two bruchids to SF fumigation regime which did not result in total mortality were also determined. The developmental period (egg laying to adult emergence) was recorded for 50 individuals emerging from eggs exposed to each SF regime. Adult emergence was observed for up to 45 days post-oviposition. The fecundity of 15 females emerging from fumigated eggs and those surviving fumigation as adults was determined by observing egg deposition by each mated female on 50 mung bean seeds in a Petri dish.

Data collected were subjected to Holm-Sidak analysis, using SigmaStat (SPSS Inc., Chicago, IL).

RESULTS

At all concentrations of SF (3.1 – 20.3 g/m^3) and temperatures (25° and 30° C) 100% mortality of developmental stages of *C. maculatus* were obtained except for the egg stage (Table 1.). At the temperature of 25° C, fumigation of the eggs with SF concentrations of 3.1 – 10.2 g/m^3 did not result in 100% mortality which was achieved with higher concentrations at the same temperature. SF concentration of 3.1 g/m^3 did not produce 100% mortality of *C. maculatus* eggs at 30° C but was recorded with 5.1 g/m^3 or higher at the same temperature.

The trend observed for *C. subinnotatus* is summarized in Table 2. At all concentrations of SF (3.1 – 20.3 g/m^3) and temperatures (25° and 30° C) 100% mortality of the developmental stages were also obtained except for the egg stage. Fumigation of the eggs with SF concentrations of 3.1 – 5.1 g/m^3 at 25° and 30° C did not result in 100% mortality but was achieved with higher concentrations.

The developmental period (egg to adult) of fumigated bruchid eggs was significantly longer than those for those which were not fumigated by 2 to 4 days (Table 3). Adults that emerged from fumigated bruchid eggs laid as many eggs ($P > 0.05$) as those emerging from eggs which were not fumigated.

There was 100% mortality of freshly emerged *C. maculatus* and *C. subinnotatus* adults fumigated with 3.1 ± 0.08 g/m^3 SF over a period of 4 or more hours at 30° C (Table 4). Whereas all the adults fumigated with the same concentration of SF over a period of 1 hour or less survived, 50% and greater than 80% mortalities were recorded for exposure for 2 and 3 hours, respectively. Adult bruchids which survived sub-lethal exposure to fumigation with 3.1 ± 0.08 g/m^3 SF laid significantly fewer 3ggs than those that were not fumigated (Table 5).

Table 1: Mortality of developmental stages of *C. maculatus* fumigated with different concentrations of SF and at two different temperatures

SF concentration g/m³	*C. maculatus* stage	Mean % corrected mortality at: 25° C	30° C	P*
3.1 ± 0.01	Adult	100.0 ± 0.00	100.0 ± 0.00	
	Egg	34.10 ± 9.10	60.5 ± 6.25	< 0.05
	Larva	100.0 ± 0.00	100.0 ± 0.00	
	Pupa	100.0 ± 0.00	100.0 ± 0.00	
5.1 ± 0.01	Adult	100.0 ± 0.00	100.0 ± 0.00	
	Egg	89.6 ± 3.31	100.0 ± 0.00	< 0.05
	Larva	100.0 ± 0.00	100.0 ± 0.00	
	Pupa	100.0 ± 0.00	100.0 ± 0.00	
10.2 ± 0.03	Adult	100.0 ± 0.00	100.0 ± 0.00	
	Egg	94.0 ± 1.81	100.0 ± 0.00	< 0.05
	Larva	100.0 ± 0.00	100.0 ± 0.00	
	Pupa	100.0 ± 0.00	100.0 ± 0.00	
15.2 ± 0.20	Egg	100.0 ± 0.00	100.0 ± 0.00	
	Larva	100.0 ± 0.00	100.0 ± 0.00	
	Pupa	100.0 ± 0.00	100.0 ± 0.00	
20.3 ± 0.19	Egg	100.0 ± 0.00	100.0 ± 0.00	
	Larva	100.0 ± 0.00	100.0 ± 0.00	
	Pupa	100.0 ± 0.00	100.0 ± 0.00	

*Holm-Sidak test

Table 2: Mortality of developmental stages of *C. subinnotatus* fumigated with different concentrations of SF and at two different temperatures

SF concentration g/m³	*C. maculatus* stage	Mean % corrected mortality at: 25° C	30° C	P*
3.1 ± 0.01	Adult	100.0 ± 0.00	100.0 ± 0.00	
	Egg	39.2 ± 0.96	71.4 ± 2.88	< 0.05
	Larva	100.0 ± 0.00	100.0 ± 0.00	
	Pupa	100.0 ± 0.00	100.0 ± 0.00	
5.1 ± 0.01	Adult	100.0 ± 0.00	100.0 ± 0.00	
	Egg	82.5 ± 3.01	93.1 ± 1.36	< 0.05
	Larva	100.0 ± 0.00	100.0 ± 0.00	
	Pupa	100.0 ± 0.00	100.0 ± 0.00	
10.2 ± 0.03	Adult	100.0 ± 0.00	100.0 ± 0.00	
	Egg	100.0 ± 0.00	100.0 ± 0.00	
	Larva	100.0 ± 0.00	100.0 ± 0.00	
	Pupa	100.0 ± 0.00	100.0 ± 0.00	
15.2 ± 0.20	Egg	100.0 ± 0.00	100.0 ± 0.00	
	Larva	100.0 ± 0.00	100.0 ± 0.00	
	Pupa	100.0 ± 0.00	100.0 ± 0.00	
20.3 ± 0.19	Egg	100.0 ± 0.00	100.0 ± 0.00	
	Larva	100.0 ± 0.00	100.0 ± 0.00	
	Pupa	100.0 ± 0.00	100.0 ± 0.00	

*Holm-Sidak test

Table 3: Developmental period (egg-adult) and fecundity of adults of *C. maculatus* and *C. subinnotatus* emerging from eggs fumigated with sub-lethal concentrations of SF for 24 hours at

SF concentration g/m^3	Mean developmental period (days) for:		Mean number of eggs laid by female emerging from fumigated eggs of:	
	C. maculatus	*C. subinnotatus*	*C. maculatus*	*C. subinnotatus*
3.1	27.4 ± 0.18b	39.3 ± 0.33b	68.4 ± 1.90a	39.5 ± 4.39a
5.1	27.6 ± 0.18b	39.9 ± 0.31b	65.1 ± 2.09a	44.3 ± 3.77a
Control	25.4 ± 0.21a	35.2 ± 0.26a	66.5 ± 3.37a	47.6 ± 3.93a

Means in each column followed by the same letters are not significantly different at P = 0.001 by Holm-Sidak test.

Table 4: Mortality of freshly emerged *C. maculatus* and *C. subinnotatus* adults fumigated with 3.1 ± 0.08 g/m^3 SF over different periods of exposure at 30° C

Exposure period (Hours)	Mean % corrected mortality of adults of:	
	C. maculatus	*C. subinnotatus*
0.5	0.0 ± 0.00a	0.0 ± 0.00a
1	0.0 ± 0.00a	0.0 ± 0.00a
2	50.0 ± 5.77c	50.0 ± 2.88c
3	85.0 ± 2.89d	86.7 ± 1.67d
4	100.0 ± 0.00e	100.0 ± 0.00e
5	100.0 ± 0.00e	100.0 ± 0.00e

Means in each column followed by the same letters are not significantly different at 5% level by Holm-Sidak test

Table 5: Fecundity of adults of *C. maculatus* and *C. subinnotatus* surviving direct sub-lethal treatment with 3.1 ± 0.08 g/m3 SF over different periods of exposure at 30° C*

Exposure period (hours)	Mean number of eggs laid by female surviving fumigation:	
	C. maculatus	*C. subinnotatus*
0.5	41.0 ± 3.18a	41.0 ± 3.18b
1	41.1 ± 5.00a	34.9 ± 2.93ab
2	32.0 ± 5.50a	25.4 ± 2.62a
Control	65.4 ± 2.92b	52.7 ± 3.18c

Means in each column followed by the same letters are not significantly different at P = 0.001 by Holm-Sidak test.

*Females which did not lay any eggs or died within 3 days after treatment were not taken into account.

DISCUSSION

The results showed that all developmental stages of *C. maculatus* and *C. subinnotatus* were killed by fumigation with 10 g/m^3 of SF at 30° C for 24 hours. The eggs of the bruchids were more tolerant to SF than the adults, larvae and pupae. This is consistent with observations reported by several other workers on the lethal effects of SF to developmental stages of other stored products insect pests particularly in the Order Coleoptera (Bell *et al*., 2003; Faruki *et al*., 2005; Baltaci *et al*., 2008). It has been observed that eggs of insect pests of different ages are variably susceptible to SF (Bell and Savvidou, 1999; Schneider and Hartsell, 1999; Baltaci *et al*., 2009), however, variation in the susceptibility of bruchid eggs with age to SF was not investigated in this study.

Complete mortality of adults, larvae and pupae of the two bruchids was achieved when fumigated with 3.1 g/m^3 or more of SF at 25° C. Guogan *et al*. (1999) reported that generally for many beetles, pupae were more resistant to SF treatment than larvae which were in turn more resistant than adults. Adult bruchids were observed in this study to be killed by the lowest SF concentration tested (i.e. 3.1 g/m^3) in just 4 hours at 30° C. It is possible that larvae and pupae of the bruchids may similarly be killed at exposure periods of less than 24 hours, but it has to be empirically verified. This way, the relative tolerance of pupae, larvae and adults of *C. maculatus* and *C. subinnotatus* to SF treatment can be accurately determined.

The results in the case of bruchid eggs have also demonstrated the modulating role of temperature in the insecticidal action of SF that has been reported by many workers (Bell and Savvidou, 1999; Bell *et al.*, 1999; Reichmuth *et al*., 1999; Bell, 2006; Baltaci *et al*., 2009). When there were survivors after fumigation of bruchid eggs with SF, mortality was always higher at 30° C than at 25° C. Thus, when for instance fumigation of *C. maculatus* eggs with 5.1 g/m^3 of SF caused 89.6% mortality at 25° C, 100% egg mortality was obtained at 30° C. Guogan *et al*. (1999) in studies in China observed 100% kill of eggs of the adzuki bean beetle, *C. chinensis* subjected to fumigation with 15 g/m^3 of SF at 20° C for 48 hours and suggested an application of 35 g/m^3 of SF at 20-24° C for 24-48 hours for the control of the pest. This is in consonance with our findings considering the fact that our studies were carried out at higher temperatures. Increased insect metabolism at higher temperatures has been suggested to elucidate their susceptibility to fumigant action at such temperatures (Price, 1985).

It was observed that the development period (egg to adult) of fumigated bruchid eggs was significantly longer than for those which were not fumigated. It has been demonstrated that the development of insect eggs does proceed, although presumably at a reduced rate during exposure to SF (Bell *et al.,* 2004). This may partly explain delayed emergence of adults from eggs fumigated with SF. Aside this delay, post-embryonic development may have occurred normally because emerging individuals did not manifest impaired reproduction. The females were as fecund as those emerging from eggs which were not subjected to fumigation.

It was further observed that the fecundity of adult females of both *C. maculatus* and *C. subinnotatus* surviving direct sub-lethal fumigation with SF was significantly reduced in comparison with females that were not subjected to fumigation. It was important that in taking the oviposition count, females which did not lay eggs at all and those that died within 3 days post-fumigation were discountenanced. Thus,

reduction of lifespan as a result of SF fumigation which was casually observed may not have played a significant role in the reduction of fecundity, since at least for *C. maculatus*, majority of the eggs are laid in the first 3 days (Credland and Wright, 1989). It has earlier been postulated that cellular energy deprivation in the insect caused by exposure to SF (Meikle *et al*., 1963), even for the putatively short duration, was sufficient to somewhat hinder reproduction and oviposition in the beetles.

It is important that SF that is being advocated as a replacement fumigant for methyl bromide is tested against all major insect pests of stored food commodities in different parts of the world. As a result of severe cross infestation which can arise from poor storage and product handling especially in developing countries, food commodities in some situations can become infested with an array of different insect species. For instance, on stored dried cocoyam chips in Nigeria, Nwana (1993) recorded more than 10 different insect species including *C. maculatus.* In such situations, the species with the highest tolerance to SF will determine the dosage for complete control of infestation.

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