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MORTALITY OF LIFE STAGES OF *CARPOPHILUS DIMIDIATUS* (F) EXPOSED TO CARBON DIOXIDE ATMOSPHERE

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ABSTRACT

Controlled atmosphere for the preservation of durable commodities has been proven effective in the control of insect infestation. This method involves the manipulation of the levels of nitrogen (N_2), carbon-dioxide (CO_2) and oxygen (O_2) gases in storage facilities to control insects and minimize their damage to the commodity. The sap beetle, *Carpophilus dimidiatus* at the egg, larval, pupal and adult stages was exposed to carbon dioxide atmosphere for 0, 2, 4, 6 and 8 hours. Post – treatment survival of the different life stages was monitored. All tests were carried out at 29±2^{0}C and 90±5% rh. An initial acclimatization period of 6 hours was required before each test. Results showed that after exposure for 8 hours to carbon dioxide atmosphere, *C. dimidiatus* eggs, larvae and pupae had 13.3%, 50.0% and 33.3% mortality respectively while the adults had 100% mortality after 6 hours of exposure. Correlation analysis between the exposure periods and mortalities shows a significant positive relationship in the larval and adult stages at 0.01 levels. Hypercarbia atmosphere had no post-treatment effect on the larvae which eclosed from exposed eggs of *C. dimidiatus*, Also exposed larvae which subsequently pupated and eclosed showed no post-treatment effect.

INTRODUCTION

Controlled atmosphere (CA) and modified atmosphere (MA) are two terms used interchangeably by various researchers (Banks, 1979; Donahaye *et al* 1996; Soderstrom *et al.*, 1996; Hodges and Surrendro, 1996). Controlling the storage atmosphere by the use of atmospheric gases is being explored since they do not display the acute lethal effects characteristic of the organic chemicals (Odeyemi and Akinnusi, 1985; Benschoter, 1987) They pose little danger to man and animals as well as presenting no residue problems in treated food commodities. The use of this technique to control insects involves the alteration of the proportions of the normal atmospheric concentrations of mainly nitrogen, oxygen, carbon dioxide and other rare gases, which make up 78%, 21%, 1.1% and 0.03% respectively of the normal

atmosphere to create an atmosphere lethal to insects (Navarro and Jay, 1987). Low levels of oxygen and high levels of carbon dioxide impose metabolic stress on insects by hindering the oxidative breakdown of a metabolic intermediate product (pyruvate) required for energy release and also causing the accumulation of a toxic product (lactic acid) (Chapman, 1971).

Most researches carried out using CA for insect control are based on achieving an increase in the carbon dioxide content of the storage environment thus producing hypercarbia atmosphere or reducing the oxygen content obtained usually by flushing with nitrogen or mixture of nitrogen and carbon-dioxide thereby producing hypoxia or anoxia atmosphere (McGaughey and Akins, 1989). In CA treatment, the lethal atmosphere must be maintained for an adequate length of time for effectiveness and thus an enclosure, which is reliably gas tight for the retention of the lethal atmosphere is required. The set up can be a continuous gas flow system (Soderstrom *et al*, 1990) or a static test system (Lindgren and Vincent, 1970; Leong and Ho, 1995). Also time of exposure or the treatment period is a critical factor (Navarro and Jay, 1987; Leong and Ho, 1995). However, the effectiveness of CA can be enhanced by varying other parameters such as temperature and relative humidity (r.h.) when the exposure period is reduced (Soderstrom *et al,* 1992 ; Mbata and Reichmuth, 1996; Ofuya and Reichmuth, 2002).

Susceptibility of stored product insects to CA varies, both between adult species and also between the various developmental stages of each insect species (Press *et al.,* 1967; Navarro and Jay, 1987; Ofuya and Reichmuth, 1998; Athie *et al.,* 1998; and Mann *et al.,* 1999). Mortality of the adult and other immature stages of such important species as *Sitophilus zeamais* Motsch., *S. oryzae* (L.), *S. granarius* (L.), *Dinoderus porcellus* , *Callosobruchus maculatus* (F.) *Rhyzopertha dominica* (F.), *Dermestes maculatus* Degeer, *Necrobia rufipes* (Degeer), *Lasioderma serricorne* (F.) and *Oryzaephilus surinamensis* (L.) have been evaluated by various researchers. The main findings derived from these researches have centered on how survival is affected by species, strain, developmental stages and pest population (Lindgren and Vincent, 1970; Odeyemi and Akinnusi, 1985; Locatelli and Daolio, 1993; Ofuya and Reichmuth, 1994).The sap beetle, *Carpophilus dimidiatus* (F.) belongs to the family Nitidulidae and the species are dependent on relatively high moisture levels in commodities for successful development and survival (Johnson, 1987). Development was 26 days at 32.5^{O}C and 90% r.h with high mortality while best survival was achieved between 22.5 and 27.5^{O}C at 70% r.h. (Haines, 1991). There is a paucity of knowledge in the use of hypercarbia atmosphere in the control of *C. dimidiatus*. Although considerable research carried out on insects infesting durables has increased knowledge on the use of different gas compositions for insect control, there is the need to further understand the sub lethal effect of these gases especially as regards post-treatment effect of exposure on test insects.

The objectives of this research were to investigate the effect of controlled atmosphere on *C. dimidiatus* adult and immature stages as well as to assess the post-treatment effect of hypercarbic condition on their survival.

MATERIALS AND METHODS

Insect culture maintenance

Cultures of *C. dimidiatus* were maintained on sterilized fresh maize cobs in 3L glass jars containing some moist soil. Exhausted cobs were continually replaced and the soil was kept moist for continuous availability of the nitidulids throughout the period of study. The jars were kept in an insectary at an ambient temperature of 30 ± 2 ^{0}C and 72 ± 10% r.h.. Pre-adult stages were fed on sterilized milled maize in Petri dishes (9 cm diameter) until adult emergence, while newly-emerged adults were confined in similar Petri dishes on milled maize.

Exposure methods

The gas tight treatment chamber was constructed of polyvinyl chloride pipe (Soderstrom et al., 1996) covered with a transparent Perspex plate. It has a removable iron hanger having steps for holding the thermo-hygrometer and the Petri dishes used in the exposure of the test insects. It has a volume of 11.23litres. This design used a Static Test System similar to the method of Leong and Ho (1995) utilizing pure CO_2 supplied from a pressurized cylinder from BOC gas (British Oxygen Company) as the purge gas. The chamber was purged when necessary to maintain an oxygen level of 1.0 ± 0.5% throughout the experimental period with a chamber temperature of 29 ± 2^{0}C and 90± 5% r.h.. The residual oxygen concentration within the chamber was measured for each experiment by withdrawing 10ml of gas sample, which is analyzed with a Toray (LF - 750) Oxygen Analyzer.

For each experiment, test insects were placed in Petri dishes covered with muslin cloth and set on the steps of the chamber iron hanger. The top step held the portable thermo-hygrometer for temperature and humidity measurement while the last step held a Petri dish containing some water soaked cotton wool for humidity control (Soderstrom et al., 1990). The loaded hanger was set in place inside the chamber and covered. An initial acclimatization period of 6 hours was allowed before each test. Each experimental batch was maintained for 2, 4, 6 or 8 hours in hypercarbia atmosphere while normal air served as control (0 hour). Each test had three replicates.

Test insects

The four life stages of *C. dimidiatus* were exposed separately to CO_2 treatment. The total number of developmental stages and age at which exposure to treatment was

carried out are shown in Table 1. The required number of eggs was collected using the method of Gbaye (2003). The exposed eggs were monitored daily for eclosion with a stereomicroscope. Hatched eggs were observed and mortality recorded. Also larval mortality was checked after treatment. Exposed pupae were removed from treatment and monitored for adult emergence while adult mortality was also recorded. Pupae that failed to emerge to adult were recorded as dead. Dead adult insects were noted and counted while the surviving ones were kept and re-checked 24 hours after being returned to normal atmosphere in case of delayed death or recovery.

Post Treatment study on exposed life stages

Further observations were made on the life stages of *C. dimidiatus* exposed to carbon dioxide atmosphere. Exposed egg dishes with eclosed larvae were monitored till subsequent pupation or death of such larvae. Likewise, exposed larvae dishes with pupae were monitored till eventual eclosion or death while exposed pupae dishes with emerged adults were monitored till mortality occurred. The exposed life stages were monitored at ambient temperature of 32 ± 2^{O}C and 90 ± 5% r.h. in the laboratory.

Data Analysis

Analysis of variance (ANOVA) was used to analyze the data and the means separated by the New Duncan's Multiple Range Test (NDMRT) (at P = 0.05). Pearson's correlation analysis was used to evaluate the relationship within the data.

RESULTS

Mortality of life stages

Table 2 shows that the mortality of the eggs of *C. dimidiatus* in hypercarbia atmosphere was very low with no significant difference ($P > 0.05$) between the exposure periods of 2 to 8 hours. At 8 hours of exposure, a mean of 13.3% of the eggs failed to hatch while at normal exposure to air (control), all the eggs hatched. The mean pupae mortality was 33.3% at 8 hours of exposure and 20.0% in the control. In the larvae, mean mortality was 85.0 % at 8 hours and 10.0% in the control while 100% adult mortality occurred at 6 and 8 hours of exposure. For all the exposure periods, there were significant differences ($P<0.05$) in larva and adult mortalities. The order of susceptibility of the life stages of *C. dimidiatus* to hypercabia atmosphere could be deduced as follows:

Egg< Pupa< Larva < Adult.

Linear regression charts reveal that there were positive correlation between the mortalities of the tested life stages and the hours of exposure to CO_2 atmosphere (Fig. 1). The Correlation Coefficients (R^2) for egg and pupae were 0.8921 and 0.9046 respectively while values for larvae and adults were 0.810 and 0.8989 respectively.

TABLE 1
Exposure of the developmental stages of *C. dimidiatus* to increased CO2atmosphere.

Developmental stage	**Number of insect stages treated**	**Age of insects (Days)**	**Developmental stage of insects**
Egg	100	0-1	Unhatched eggs
Larva	50	3-5	Larvae from eggs
Pupa	50	2-4	Pupae from pupation
Adult	50	4-6	Adults from emergence

Linear regression charts showing correlation between the mortalities of the tested life stages and the hours of exposure to CO_2 atmosphere

TABLE 2
Mortality of *C. dimidiatus* exposed to CO_2 atmosphere at 29 ± 2^0C and 90 ± 5% r.h. for varied periods.

Exposure period (hours)	**Percentage mortality (mean ± S.E)**			
	Egg	**Larva**	**Pupa**	**Adult**
0 (Control)	0.0±0.00a(a)	10.0±4.09a(a)	20.0± 8.17a(a)	0.0±0.00a(a)
2	6.7±2.36a(a)	60.0±7.07b(b)	20.0±4.08a(ab)	40.0±4.08b(b)
4	6.7±4.72a(a)	66.7±8.50b(b)	26.0±4.72a(ab)	40.0±8.17b(b)
6	13.3±6.24a(a)	83.3±6.24c(cb)	26.7±4.72a(a)	100.0±0.00c(c)
8	13.3±4.72a(a)	85.0±10.80c(c)	33.3±9.43ab(b)	100.0±0.00c(c)

Mean values followed by the same letter(s) are not significantly different (P≥ 0.05) by New Duncan's Multiple Range Test. Letters immediately following the means are for vertical comparison whilst letters in parenthesis are for horizontal comparison.

Post treatment effect on *C. dimidiatus*

Table 3 shows the mean development period from egg to larva, larva to pupa and pupa to adult stages in *C. dimidiatus* after exposure to carbon dioxide gas

concentrations. Results revealed that at ambient temperature (32 ± 2^{O}C) and r.h. (90 ± 5%) in the laboratory, development of the life stages was not affected, though the period for development required by the stages of treated beetles was longer than that required by the untreated control. The mean period for hatching of eggs was 2.5 days in the control while period for hatching was 5.0 days at 6 hours and 6.6 days at 8 hours of exposure. Pupation of larvae was 10.0 days at control while it was 12.6 days at 6 hours and 16 days at 8 hours of exposure. Adult emergence was 8.0 days at control while it was 12.0 days at 6 hours, and 16.0 days at 8 hours. However, adult life span was 76.0 days at control while it was 64.2 days at 2 hours and 57.0 days at 4 hours of exposure only. This shows that length of days for adult *C. dimidiatus* development was affected by exposure to high carbon dioxide gas concentrations.

DISCUSSION

This study on the development of *C. dimidiatus* in carbon dioxide atmosphere was carried out on the egg, larval, pupal and adult stages. There is little information on the response of this beetle to hypercarbia atmospheres.

The egg stage of *C. dimidiatus* was observed to be the most tolerant life stage to carbon dioxide gas. Similarly, the egg stage of various storage insect pests such as *Oryzaephilus surinamensis, Galleria mellonella* (L.) and *Callosobruchus maculatus* (F.) have also been reported to be tolerant to hypercarbia atmospheres than the other life stages (Locatelli and Daolio, 1993; Donahaye *et al.*, 2000; Ofuya and Reichmuth, 2002). At normal oxygen level, Emekci *et al.* (2001) observed that egg respiration in *Tribolium castaneum* (Herbst) was lower than other stages. Chapman (1971) reported that most insect eggs have less oxygen requirement than other life stages followed by the pupae, which as the quiescent stage, might require less oxygen for survival. He further stated that the tolerance of the eggs could also be due to the relative impermeability of the egg chorion to gases when compared to the body surface and integuments (with spiracles) of other life stages.

TABLE 3
Effect of the exposure of four stages of *C. dimidiatus* to CO_2 at29± 2^{O}C and 90± 5% rh. on the period of development.

Developmental stages	**Mean period of development (Days)**				
	0 (Control)	**2 hours**	**4 hours**	**6 hours**	**8 hours**
Egg-larva	2.5	3.2	4.2	5.0	6.6
Larva -pupa	10.0	10.9	11.5	12.6	16.0
Pupa-adult	8.0	9.2	10.0	12.0	16.0
Adult life span	76.0	64.2	57.0	*	*

In this study, the longest CO_2 treatment used was 8 hours and the egg hatchability obtained was not significantly different from those of the shorter periods and the control. However, a study by Locatelli and Daolio (1993) showed that increasing the exposure period would subsequently increase the egg mortality significantly. They recommended an exposure period of 18 hours in carbon dioxide atmosphere for the control of *O. surinamensis* egg and 24 hours each for the eggs of *Rhyzopertha dominica* and *Sitophilus oryzae* at 30^{O}C. Also the high humidity (90± 2%r.h.) used in this study might have reduced the egg mortality. Ofuya and Reichmuth (2002) likewise recorded significantly lower mortality of eggs of *C. maculatus* in 70% $C0_2$ in air at higher r.h. (90 ± 3%) than at lower r.h. (34 ± 2%). Chapman, (1971) reported that insect activity, which results in a sharp increase in oxygen consumption, is used, as a measure of metabolism and this is usually greater in adults and larvae than in pupae and eggs. In this study, high adult and larval mortalities were observed in comparison to the egg and pupal life stages of *C. dimidiatus*. Navarro and Jay (1987) reported the same observation on the adults of *S. oryzae, T. castaneum* and *O. surinamensis* when their life stages were exposed to high concentrations of carbon dioxide. This is probably due to low levels of oxygen and high carbon dioxide, which hinders the oxidative breakdown of pyruvate that is required for energy release from food. This condition also causes the accumulation of lactic acid that is toxic to all post embryonic stages. Excessive carbon dioxide is known to be poisonous to insects causing death (Fullick, 1994; Odeyemi and Daramola, 2000).

The mortality trend observed in this study was Egg < Pupa < larva < adult. Positive correlation between percentage of mortality and period of exposure to CO_2 was observed in all the life stages. This is similar to the trend reported by Locatelli and Daolio (1993) in the life stages of *R. dominica, S. oryzae, O. surinamensis* and *Plodia interpunctella* (Hubner). Donahaye *et al*. (1996) reported 95% mortality for larval, pupal and adult stages of two nitidulid beetles after 196 hours exposure to a gas concentration of 3% O_2, 85% N_2 and 12% CO_2 at 26^{O}C. They also estimated that the same level of mortality should occur after 60 hours of exposure to a gas concentration of 1% O_2, 85% N_2 and 14% CO_2. at 35^{O}C. This variation in species susceptibility to controlled atmosphere among storage insects of the same genus had been reported earlier (Lindgren and Vincent, 1970; Leong and Ho, 1995). Also the type of gas used could affect the mortality of these insects. Studies have shown that purging a treatment chamber with CO_2 is more effective and more deleterious to storage insects (Press *et al.,* 1967; Hooper, 1970; Fleurat-Lessard, 1987; Navarro and Jay, 1987; Soderstrom *et al*., 1990; 1996; Ofuya and Reichmuth, 1998).

Dawson (1995) stated that even a brief exposure of insects to high concentration of CO_2 could have both short term and long term effects. He cited such effect in an increased mortality in *Drosphila melanogaster* (Meigen) and reduced fecundity in both *D. melanogaster* and *C. maculatus* anaesthetized by CO_2. Hooper (1970) reported the same effect on *Ceratitis capitata* (Wiedman). It was observed in

this study that exposure of the developmental stages to high CO_2 concentrations prolonged the period of egg hatching and pupation in *C. dimidiatus*. However the adult life span was shortened with high CO_2 concentrations. This indicated that the larvae and pupae stages carried the high CO_2 effect into their adult life where this was well manifested. Lefkovitch (1966) and Haines (1991) reported an average life span of 3 months for adults of *C. dimidiatus* at favourable conditions. Chapman (1971) reported that when carbon dioxide accumulates in insect blood, it decreases the pH and only diffuses slowly from the blood to the gaseous phase. The extended high acidity of the blood could affect the osmotic balance in the insect system and this could have led to the subsequent death of the emerged adult even in normal atmosphere.

CONCLUSION

This study reveals that carbon dioxide gas was most effective on the larva and adult stages of *C. dimidiatus*. Exposure to carbon dioxide prolonged the period of egg hatching, pupation and adult emergence while the life span of adult *C. dimidiatus* was reduced considerably. However exposure of the egg and pupal stages of *C. dimidiatus* to longer periods of carbon dioxide could increase their mortality. Thus further studies are required on the oviposition deterrent, fecundity and productivity of the life stages of *C. dimidiatus* after exposure to carbon dioxide.

REFERENCES

Athie, I., Gomes, R.A.R., Bolonhezi, S., Valenentini, S.R.T. and De-Castro, F. P. (1998) Effects of carbon dioxide and phosphine mixture on resistant populations of stored grain insects. *Journal of Stored Products Research* **34,** 27-32.

Benschoter, C.A. (1987) Effects of Modified Atmosphere and refrigerationtemperatures on survival of eggs and larvae of the Carribean fruit fly (DipteraTephritidae) in laboratory diet. *Journal of Economic Entomology* **80,** 1223-1225.

Banks, H. J. (1979) Recent Advances in the use of modified atmospheres for storedproduct pests control. In Proceedings of International Working Conference on Stored Product Entomology, Ibadan, Nigeria. pp 198-217.

Chapman, R. F.(1971) The Insects :Structure and Function. 2ndEdition. Hodder andStoughton Ltd, London, 819pp.

Dawson, C. (1995): The effect of carbon dioxide induced anesthesia on fecundity of Callosobruchus maculatus (Coleoptera: Bruchidae). *Journal of Stored ProductsResearch,* **31,** 49-54.

Donahaye, E., Navarro, S. and Rindner, M. (1994) The influence of temperature onthe sensitivity of two nitidulid beetles to low oxygen concentration. In: Proceedings of the 6th International Working Conference on Stored Product Protection 1: 88-90

Donahaye, E. J., Navarro, S., Rinder, M. and Azrieli, A.(1996) The combined influence of temperature and Modified Atmospheres on Tribolium castaneum (Herbst) (Coleoptera: Nitidulidae). *Journal of Stored Products Research* **32,** 225-232.

Donahaye, E. J., Navarro, S., Rinder, M. and Azrieli, A. (2000) Sensitivity of the greater wax moth, Galleria mellonella to carbon dioxide enriched Modified Atmosphere. In : *Proceedings of the 7th International Working Conference on Stored Product Protection*, **1**: 692-696.

Emekci, M., Navarro, S. Donaye, J. E., Rindner, M. and Azrieli A. (2001) Respiration of Stored Product pests in Hermetic conditions. In: Donahaye, E.J., Navarro, S. and Leesch, J. (Eds). *Proceedings of an International Conference on Controlled Atmosphere and Fumigation in Stored Products*, Fresno, CA. 29th October- 3rd November 2000, Executive Printing Services, Clovis, CA, U.S.A. pp. 26-35.

Fleurat Lessard, F., (1987) Control of storage insects by physical mean and modifiedenvironmental conditions: Feasibility and applications. In: Lawson, T. J. (ed) Stored Products Pest Control. British Crop Protection Council, Monograph **37**: 209-218.

Fullick, A. (1994) Respiratory systems. In: Heinemann Advanced Science Biology. Heinemann Educational Publishers, Oxford. pp. 121-133.

Gbaye, O. A. (2003) The use of controlled atmosphere for the control of Carpophilus spp. (Coleoptera: Nitidulidae) and Drosophila melanogaster (Meigen) (Diptera:Drosophilidae). M. Tech. Dissertation, Federal University of Technology,Akure. pp 64.

Haines, C. P. (1991) Insects and Arachnids of Tropical Stored Products: Their Biology and Identification (A Training Manual). Natural Resources Institute,Chatham,U.K. 180pp.

Hodges, R. J. and Surrendro (1996) Detection of controlled atmosphere changes in CO2 flushed sealed enclosures for pest and quality management of bagged milled rice. *Journal of Stored Products Research* **32,** 97-104.

Hooper, G. H. S. (1970) Use of carbon dioxide, nitrogen and cold to immobilize adults of the Mediterranean fruit fly. *Journal of Economic Entomology* **63**: 1962-1963.

Johnson, J. A. (1987) Sensitivity of larvae, pupae and adults of the dried fruit beetle (Coleoptera: Nitidulidae) to gamma radiation. *Journal of Economic Entomology* **80,** 1302-1305.

Lefkovitch, L. P. (1966) Some observations on the life cycle of Carpophilus dimidiatus (F) (Coleoptera: Nitidulidae) on wheat bran. *Journal of Stored Products Research* **2 ,** 163-165.

Leong, E. C. W. and HO, J. H. (1995) Effects of carbon dioxide on the mortality of Liposcelis bostrychophila Bad. and Liposcelis entomophila (End.) (Psocoptera: Liposcelididae). *Journal of Stored Products Research* **31**, 185-19.

Lindgren, D. L. and Vincent, L. E. (1970) Effect of atmospheric gases alone or in combination on the mortality of granary and rice weevils. *Journal of Economic Entomology* **63,** 1926-1929.

Locatelli, D. P., and Daolio, E. (1993) Effectiveness of carbon dioxide under reduced pressure against some insects infesting packaged rice. *Journal of Stored Products Research* **29**, 81-87.

Mann, D. D., Jayas, D. S., White, N. D. G. and Muir, W. E. (1999) Mortality of adult Cryptolestes ferrugineus (Stephens) exposed to changing CO2 concentrations. *Journal of Stored Products Research* **35**: 385-395.

Mbata, G. N. and Reichmuth, C. (1996): The comparative effectiveness of different Modified Atmospheres for the disinfestations of bambarra groundnuts, Vigna subterranean(L) Verde, infested by Callosobruchus subinnotatus (Pic) (Coleoptera: Bruchidae). *Journal of Stored Products Research* **32**, 45-51.

McGaughey, W. H. and Atkins, R. G. (1989): Application of Modified Atmospheres in farm grain storage bins. *Journal of Stored Products Research* **25**, 201-210.

Navarro, S. and Jay, E. G. (1987) Application of Modified Atmospheres for controlling stored grain insects. In: Lawson, T. J.(Ed.).Stored Products Pest control. British Crop Protection Council, Monograph **37**, pp.229-236.

Odeyemi, O. O. and Daramola, A. M. (2000) Storage Practices in the Tropics. Volume 1.Food Storage and Pest Problems (1st ed). Dave Collins PublicationNigeria.253pp.

Odeyemi, O. O. and Akinnusi, O.A. (1985) Effects of nitrogen atmosphere on the adults and immature stages of some stored product insects. *Nigerian Journal of Entomology* **6**: 39-42.

Ofuya, T.I. and Reichmuth, C. (1994) Effect of level of seed infestation on mortality of larvae and pupae of Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) in some Controlled Atmospheres. *Journal of Stored Products Research* **30,** 75-78.

Ofuya, T.I. and Reichmuth, C. (1998) Effect of combining or alternating hypercabia and anoxia conditions on mortality of two bruchids infesting grain legumes in storage. In: Lale, N. E. S., Molta, N. B., Donli, P. O., Dike, M.C. and Aminu-Kano, M.(Eds.)1998. Entomology in the Nigerian Economy: Research Focus in the 21st century. Entomological Society of Nigeria (ESN) Occasional Publications 31:221-226.

Ofuya, T.I. and Reichmuth, C., (2002) Effect of relative humidity on thesusceptibility of Callosobruchus maculatus (Fabricius) (Coleoptera: Bruchidae) to two modified Atmospheres. *Journal of Stored Products Research* **38:** 139-146.

Press, A. F., Marzke, F. O. and Perman, G. C. (1967) Mortality of Trogodermaglabrum larvae in simulated wheat storages purged with CO_2 or N_2. *Journal of Economic Entomology* **60,** 415-416.

Soderstrom, E. L., Brandl, D. G. and Mackey, B. (1990) Responses of codling moth (Lepidoptera: Tortricidae) life stages to high carbon dioxide or low oxygenatmospheres. *Journal of Economic Entomology*. **83,** 472-475.

Soderstrom, E. L., Brandl, D. G. and Mackey, B. (1992): High temperature combined with carbon dioxide enriched or reduced oxygen atmosphere for control of Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). *Journal of Stored Products Research* **28,** 235-238.

Soderstrom, E. L., Brandl, D. G. and Mackey, B. (1996): High temperature andControlled Atmosphere treatment of codling moth (Lepidoptera: Tortricidae) infested walnuts using a gas–tight treatment chamber. *Journal of Economic Entomology* **89,** 712-714.