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Effect of Grain Breakage and Relative Humidity on The Development of *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) Infesting Stored Maize in Lafia, Nigeria

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Abstract
The effect of five levels of grain breakage at 20, 40, 60, 80 and 100% of 10g grain with control (unbroken grains) were studied on the development of Cryptolestes ferrugineus (Stephens) (Coleoptera: Laemophloeidae) under ambient laboratory conditions in Lafia, (08.33^{0}N and 08.32^{0}E) Nasarawa State Nigeria. In another set up of experiment, different levels of relative humidity generated from boxes measuring 20 x 20 x 20 cm fitted with electric light bulbs with range of 100, 60, 45, 25 watts with Control (no light bulb) were studied to determine the effect of relative humidity on the development of C. ferrugineus. These studies were laid out in a complete randomized design and replicated three times. Data was collected on number of adult mortality and progeny emergence for 20 and 56 days, respectively for both studies following date of infestation. Results of the different levels of grain breakage showed that there was significant difference in the level of grain breakage and the control treatment ($P \leq 0.05$). Results showed that maize grains broken at 80 and 100%/10 g of grain had more significantly adult emergence of C. ferrugineus when compared to other levels of grain breakage. The effect of relative humidity on progeny emergence showed that there was significant effect of relative humidity on the population and emergence (development) of C. ferrugineus. Different exposures to RH on C. ferrugineus increased insect mortality as relative humidity decreased. Mortality of up to 100% of C. ferrugineus was recorded at lower relative humidity when compared with the mortality in treatments having lower levels of RH as no emergence was recorded at RH of 18 and 24%. Treatments with high RH recorded high number of emergence as observed at 41 and 47% R.H. while 35% R.H. had the lowest adult emergence. The results of correlation among RH, temperature and mortality of adult C. ferrugineus showed that there were highly significant ($P \leq 0.01$) but negative correlations among relative humidity and temperature and mortality of adult C. ferrugineus. The potential of storing grains in low relative humidity environment coupled with low grain breakage is advocated for the management of C. ferrugineus.
***Key words**: Cryptolestes ferrugineus, Maize grain, Grain breakage, Relative-humidity, Insect mortality*

Introduction

Most cereal crops are not completely consumed after harvest as they are stored for various reasons. The problem farmers' face in Nigeria is infestation and damage caused by insect pests such as, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae), *Rhyzopertha dominica* Fab. (Coleoptera: Bostrichidae), *Prostephanus truncatus* Horn. (Coleoptera: Bostrichidae) when stored as whole grain (Hill, 2001). On the other hand, threshed or milled maize products are often attacked by secondary pests in the family Tenebrionidae, Cucujidae and Laemophloeidae (Haines, 1991). Maize grain is often threshed after winnowing before it can be used at homestead level. The method often employed for threshing maize grains at the homestead level involves spreading of

maize cobs on the ground on a tarpaulin and in some cases the cobs are put in a jute bag and tied up and thereafter beat up with a stick or pestle to separate grains from the cob. These threshing processes cause maize grain to break or crack and thence pre-dispose the grains to various stored product insect pests if not properly stored. Two major pests of threshed maize (broken grains) are the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Cryptoletes* spp. (Ajayi and Rahman, 2006). These two insect pests are secondary pests as they cannot initiate infestation on their own except on broken or milled grains. *Cryptolestes* species have been reported to breed rapidly on milled produce or on grains previously damaged by insects or by poor harvesting, storage or handling. The presence of *Cryptoletes* spp. on any cereal produce leads to grain heating, thus causing caking, presence of exuviae, frass and bio-chemical changes in the quality of grains (Haines, 1991). However, *C. ferrugineus* (Flat Grain Beetle) can attack apparently intact grain with damage caused by harvesting and handling being sufficient to facilitate entry.
Various climatic conditions are responsible for the normal reproduction, production of eggs as well as egg hatchability of insects. Relative humidity (R.H.) is a major factor that can affect the physiology and thus the development, longevity and oviposition of many insects. At low relative humidity, insect development may be retarded; while at high relative humidity or in saturated air (100% R.H.) insects or their eggs may be drown or be infected more readily by pathogens (Gullan and Cranston, 2005). Different exposures of relative humidity on stored product pests such as *Tribolium castaneum*, *T. confusum* (Coleoptera: Tenebrionidae) *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) and *C. ferrugineus* have been reported to show increased insect mortality as relative humidity decreased (Lale, 1992). Low relative humidity can prevent embryo development and egg hatching due to loss of lubrication and cuticular softness in insect (Gullan and Craston, 2005). High relative humidity contributes to population increase in stored product pest, as shown in *C. ferrugineus*, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) under laboratory conditions (Ouedraogoa *et al*., 1996). *C. ferrugineus* is one of the stored product pests that its eggs hatchability and population growth rate is primarily affected by grain temperature and relative humidity (Hagstrum, 1989; Anon., 2009). The present study was therefore, undertaken to access the effect of different levels of maize grain breakage and relative humidity on the biology of the Flat Grain Beetle, *Cryptolestes ferrugineus* (Steph.) (Coleoptera: Laemophloeidae**).**

Materials and Methods

Insect Rearing and Maintenance

The initial stock of the Flat Grain Beetle (FGB) (*C. ferrugineus*) used for the study was obtained from an untreated maize seeds purchased from Lafia Market (08.33^{o}N and 08.32^{o}E) Nasarawa State, Nigeria. The obtained insect was cultured for three months in the laboratory of the Department of Agronomy, Faculty of Agriculture (Shabu-Lafia Campus), Nasarawa State University, Keffi on maize grains that were pulverized with a pestle and mortar. From this stock, new cultures were reared in the laboratory on new maize seeds but were pulverized as described above at ambient temperature and relative humidity before they were used for the experiment.

Preparation of Maize Seeds for the Study

Three kilograms of pristine maize (var. Makera) was purchased from a local market in Lafia. The maize grains were refrigerated at sub-0^{o}C for 5 days in order to ensure that there was no surviving insect in the maize seeds. The seeds were later air dried for three days in the laboratory under screen as a protector in other to equilibrate the seeds with the conditions in the laboratory. The moisture content of the maize seeds was determined using hot air oven at a temperature of 105^{0}C for 24 hours by weighing the initial weight of the maize grain before putting into the oven after which the final weight was taken after 24 hours. The moisture content was determined using the equation

$$\% \text{ M.C} = \frac{\text{weight of sample - weight of oven dried sample}}{\text{Weight of oven dried sample}} \times 100$$

Preparation of the Temperature and Humidity Chamber

The boxes were constructed using wooden materials which were made into a cubic shape of 20x20x20 cm in size. Each of the boxes was connected with an electric lamp holder inside the top of the lid. The electrical wiring was done by connecting each box with each other for ease of control through switch of on and off. A plastic Petri-dish of 100 ml was placed in each of the boxes in which 50 ml of distilled water was added as this provided the needed humidity to the boxes through the effect of the temperature generated by the electrical bulbs.

Effect of Level of Grain Breakage on the Development of *C. ferrugineus*

From the equilibrated and stored maize grains, 10g each of the grain was weighed into twenty four 50 ml glass jars. Each 10g maize grain sample was pulverized gently with a laboratory pestle and mortar to obtain five levels (treatments) of grain breakage 20, 40, 60, 80, and 100% grain breakage. This was obtained by weighing and crushing 2, 4, 6, 8 and 10g, respectively from the 10g maize grains. The control treatment consisted of 10 g of whole and unbroken (0%) maize grains. Five pairs of unsexed adult *C. ferrugineus* were introduced into each jar with the aid of an aspirator. The glass jars were then covered with lids having ten equidistant holes of 0.05 x 0.05 mm. Thereafter, the beetles were allowed to mate and oviposit for 20 consecutive days in each glass jar. After 20 days all dead or live insects were removed from each treatment. The glass jars were checked daily for adult emergence and the number emerging in each treatment was recorded for 20 consecutive days. At each time of observation, all adults that emerged were removed from each treatment. The experiment was set up under laboratory conditions in a complete randomized design with four 4 replications per treatment.

Effect of Relative Humidity and Temperature on the Development of *C. ferrugineus* Infesting Damaged Maize

Maize seeds comprising of 20% (broken grains) and 80% (unbroken grains) (i.e. 8 g of unbroken grains mixed with 2 g of broken grains) was weighed into sixty 50 ml glass jar bottles using an electronic laboratory scale balance. Thereafter, five pairs of unsexed adult *C. ferrugineus* were introduced into each 50 ml bottle jar and covered at the mouth with its lid. 50 ml of distilled water was added into a100 ml crucible and this was placed in each test box of 20x20x20 cm. Each treatment consisted of boxes fitted with the following range capacity of electric light bulb (RH_1 = 100 watt, RH_2 = 60 watt, RH_3 = 40 watt, RH_4 = 25 watt and RH_5 = Control (in which case electric light bulb and distilled water in a crucible were not added), with the temperature generated by the electric light bulb in the boxes was measured with the aid of a mercury thermometer inserted into each box. The beetles were allowed to mate and oviposit for 20 days in the glass jars and after 20 days all dead and live insects were removed and thereafter checked daily for adult emergence and the number of emerging adults in each treatment was recorded on weekly basis. All adults that emerged were removed from each treatment at the point of observation. The experiment was laid out in a complete randomized design and replicated three times.

Data Collection and Analysis

Data collected included the number of dead and number of emerging adults in each treatment. The different temperature regimes as it relates to the energy generated by each wattage of the different electric light bulbs fitted in the boxes; the relative humidity in each box as relates to the amount of distilled water used/added to the crucible in each treatment with relative to the wattage in each boxes were analyzed using the following formula, Y= -2.871x+127.0 according to Sheeba and Alka (2011). Where: Y = relative humidity (%); x = air temperature of the enclosure in the boxes. The data collected was subjected to analysis of variance (ANOVA). All percentage data were arc sine transformed and differences between means were compared using the least significant difference (LSD) test at P=0.05. Correlation analysis was carried out to show the association between relative humidity, temperature and mortality of *C. ferrugineus*. The data was analyzed using Genstat Discovery Edition (2007).

Results

Table 1 represents the mean percentage mortality of adult *C. ferrugineus* after 20 days following date of infestation on different levels of maize grain breakage. The Table shows that there was significant mortality difference (P≤0.05) between the numbers of adults in the different levels of grain breakage and the control. It was observed that there were significant (P≤0.05) differences between the percentage of adults that died in 20 and100%/10 g of maize grain breakage and the control. Mortality of adults *C. ferrugineus* were however, statistically similar (P=0.05) in maize grains broken at 10, 60, 80 and 100%/10g of maize levels. High number of adult mortality was recorded in the control (no breakage) when compared with 80 and 100%/10g of grain breakage.

Table 1: Percentage Mortality of Adult *C. ferrugineus* on Different Levels of Maize Grain Breakage after 20 Days of Infestation.

Percentage breakage/10 g maize grain	R1	R2	R3	R4	Mean
20	30 (33.21)*	70 (56.70)	20 (26.56)	30 (33.21)	37.40 (38.70)
40	20 (26.56)	30 (33.21)	40 (39.23)	40 (39.23)	34.60 (36.03)
60	50 (45.00)	40 (30.23)	20 (26.56)	20 (26.50)	34.40 (35.91)
80	00 (0.00)	00 (0.00)	40 (39.23)	50 (45.00)	21.10 (26.97)
100 (whole breakage)	20 (26.50)	10 (18.44)	10 (18.44)	00 (0.00)	15.90 (23.50)
Control (no breakage)	60 (50.77)	80 (63.44)	70 (56.79)	20 (26.56)	49.40 (44.66)
SED					11.04
LSD(0.05)					23.54

*Values in parenthesis are arc sine transformed values to which SED and LSD values are applicable.

Table 2 shows that in the first, second and third weeks following 20 days after infestation, there were significant differences (P≤0.05) in the number of adult beetle emergence between the levels of the grain breakage. In the first week, there was no significant difference (P≥0.05) in the number of adult emergence in between the levels of grain breakage, except the number observed in emergence between 100% unbroken grain and control (no breakage) that was statically significant (P≤0.05) and different from each other. By the second week however, it was observed that there was more number of adult emergence in 80 and 100%of grain breakage when compared with other levels of grain breakage but these were not statically different (P≥0.05) from each other but were significantly different (P≤0.05) from adult emergence in 20, 40, 60% and control in which there was no emergence. Emergence of adult *C. ferrugineus* in the third week followed the same trend as was observed in the second week.

Table 2: Effect of Level of Maize Grain Breakage on Number of Emerged Adults of *C. ferrugineus* After a Three Week Period

Percentage breakage/ 10 g grain	Mean number of emerged adults after 3 weeks		
	1st	**2nd**	**3rd**
20	0.50	0.75	1.00
40	0.75	1.25	1.50
60	1.50	2.00	1.75
80	1.50	2.50	3.25
100 (whole breakage)	2.00	3.75	3.75
Control(no breakage)	0.00	0.00	0.00
SED	1.29	1.78	1.07
LSD(0.05)	1.61	2.23	1.34

Table 3 presents the effect of relative humidity on the biology of *C. ferrugineus* after 20 days following date of infestation infesting damaged stored grains. The Table shows increase in insect mortality as relative humidity decreases. Relative humidity significantly has effect on the population of *C. ferrugineus* after 20 days following date of infestation. There were significant differences ($P \leq 0.05$) between the relative humidity thereby causing mortality to adult *C. ferrugineus*. Relative humidity 1, 2 and 5 were significantly different from the other relative humidity while relative humidity 3 and 4 are statistically similar ($P=0.05$). It was observed that high mortality rates were recorded in relative humidity 1 and 2 of up to 100%, respectively with relative humidity 4 having no record of mortality and less mortality were recorded in relative humidity 3 and 5.

Table 3. Mean Number Mortality of Adult *C. ferrugineus* on Broken Maize Grain at Different Relative Humidity as Affected by Temperature Range During Storage

Relative Humidity (%)	Temperature	Mortality	Mortality Transformed $\sqrt{(x+1)0.5}$
18 (T_1)	38^0C	10	3.16
24 (T_2)	36^0C	9.33	3.05
35 (T_3)	32^0C	0.25	0.25
41 (T_4)	30^0C	0.0	0.0
47 (T_5)	28^0C	0.92	0.82
	Mean	4.10	1.46
	Significance	< 0.001***	<0.001***
	SEM	0.146	0.091
	LSD	0.414	0.257

Table 4 presents the number of adult *C. ferrugineus* emerged from broken maize grains at different levels of relative humidity from week 1 to week 8 after 20 consecutive days following date of infestation. Table 4 presents the number of adult *C. ferrugineus* emerged from broken maize grains at different levels of relative humidity from week 1 to week 8 after 20 consecutive days following date of infestation. The results showed that at lower RH of 18 and 24%, no adult *C. ferrugineus* was observed. More adult insects emerged during the first week infestation at 35, 41 and 47% RH; and these were all significantly different from each other (P<0.001). As the period of infestation increases from second to eight weeks, lower number of adult *C. ferrugineus* emerged from the different levels of RH (35, 41 and 47%%), respectively and were all significantly (P<0.001) from each other.

The results of the correlation analysis showed the type and magnitude of associations between relative humidity, temperature and mortality of *C. ferrugineus* as presented in Table 5. Results revealed that there were highly significant (P≤0.01) but negative correlations between relative humidity and temperature and also on mortality of adult *C. ferrugineus*. However, temperature and mortality of adult *C. ferrugineus* were found to be highly significant (P≤0.01) and positively correlated. More so, highly significant (P≤0.01) and positive correlation was observed between the actual mortality and transformed mortality of adult *C. ferrugineus*.

Table 4. Mean Number of Adult Emergence of *C. ferrugineus* from Broken Maize Grains from Week 1 to Week 8 After 20 Days Following Date of Infestation

Relative Humidity (%)	Week 1 Emergence	Week 2 Emergence	Week 3 Emergence	Week 4 Emergence	Week 5 Emergence	Week 6 Emergence	Week 7 Emergence	Week 8 Emergence
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
35	41.2	16.4	20.33	15.42	10.17	11.83	8.08	5.6
41	48.8	9.2	15.83	13.50	6.33	8.17	8.00	14.0
47	25.3	5.9	17.67	12.00	8.67	2.08	4.08	3.8
Mean	25.1	6.3	10.77	8.18	5.03	4.42	4.03	4.7
Significance	<0.001	<.001	<.001	<.001	<.001	<.001	<.001	0.001
SEM	3.89	2.34	2.22	1.84	1.35	0.94	1.01	2.50
LSD	11.03	6.65	6.30	5.21	3.82	2.66	2.86	7.09

Table 5: Matrix of Correlation Analysis Showing Association Between Relative Humidity, Temperature and Mortality of *C. ferrugineus*

Variables	Relative Humidity	Temperature	Mortality	Mortality Transformed
Relative Humidity	1			
Temperature	-1.000**	1		
Mortality	-0.906**	0.914**	1	
Mortality Transformed	-0.858**	0.866**	0.991**	1

Discussion

C. ferrugineus is a worldwide cosmopolitan pest of stored products; particularly grains stored as grits and behave as secondary pests following infestation of primary stored grain pests such as *Sitophilus spp*. or *Rhizopertha dominica* (Tuff and Telford, 1964). In a related study, Lale and Yusuf (2001) also showed that whole grains of pearl millet were significantly more resistant to infestation by *T. castaneum* than broken grains. Earlier reports by Li and Arbogast (1991) showed that *C. ferrugineus* exhibited high affinity for cracked maize grain than undamaged maize. The intact testa of grains has been reported to serve as mechanical barriers against infestation of whole grains by secondary pests of stored grain (Lale and Yusuf, 2001). Campbell and Runnion (2003) had reiterated the fact that *C. ferrugineus* is a polyphagous cosmopolitan pest that feeds and thrives on varieties of broken grain. Damaged grain has been reported to release some volatile compounds and these facilitate the attraction of secondary pest by broken grains (Tremattera *et al.,* 2000). The inability of *C. ferrugineus* to have population surge as the generation period increased could be due to the type of diet used as substrate. Different workers had reported that quality of diet presented to secondary pests plays a major role in their development and population abundance (Lale *et al.,* 2000; Ajayi and Rahman, 2006). There is also possibility of cannibalism and predation by both adult and larvae of *C. ferrugineus* due to the volume of substrate present to the test insect (Suresh *et al.*, 2001; Mason, 2003). None of these factors were looked into this study; however, the results obtained from this study showed that damaged grains facilitated the attraction of *C. ferrugineus.*

Relative humidity has significant effect on the population and emergence (development) of *C. ferrugineus* at different exposure of relative humidity. Development of *C. ferrugineus* showed increased in mortality as relative humidity decreased as seen in Table 3. Mortality of up to 100% of *C. ferrugineus* was recorded at lower relative humidity when compared with the mortality in relative humidity having lower levels of relative humidity. Gullan and Cranston (2005) reported that various climatic conditions are responsible for the abnormal reproduction, production of eggs as well as egg hatchability of insects. Relative humidity can affect the physiology and thus the development, longevity and oviposition of many insects (Gullan and Cranston 2005). At low relative humidity, development may be retarded, as seen in Table 4. Treatments with high relative humidity recorded high number of emergence of *C. ferrugineus*. However, low relative humidity has been reported to prevent embryo development and egg hatching due to loss of lubrication and cuticular softness in insects (Guarneri *et al*. 2002). According to Ouedraogoa *et al.* (1996) it has been reported that high relative humidity contributes to population increase in stored product pest's particularly *C. ferrugineus* development. Female *C. ferrugineus* showed higher fecundity and longer adult lifespan at high humidity. High humidity support reproductive capacity in insects as percentage of water is correlated with the amount of fat (including eggs in females), which consists of anhydrous molecules, and with the amount of cuticle, which has a lower water content than other tissues. Environmental temperature and humidity affects the developmental phase and transpiration through insect body surface (Chapman, 1998; Guarneri *et al*., 2002). It has been reported that insect survival is influenced by its ability to tolerate fluctuations in body water maintenances influenced by relative humidity (Romoser and Stoffolano, 1998). Insects therefore, must keep body water content within certain limits which is influenced by the degree of the insect cuticle permeability (Willmer, 1982; Raghu *et al*., 2004). *C. ferrugineus* is one of a number of insect pests of stored grains where moisture content requirement is higher than normal. Eggs hatchability and population growth rate is primarily affected by grain temperature and relative humidity (Anon, 2009).

The correlation coefficient is the measure of degree of symmetrical association between variables and helps us in understanding the nature and magnitude of association among relative humidity, temperature and mortality of *C. ferrugineus*. Several researchers have attempted to determine how climatic conditions contribute to the population increase in stored product pest (Quedraogoa *et al*., 1996). The significant positive and negative correlations that were observed between relative humidity, temperature and mortality of *C. ferrugineus* could be explained by the fact that, relative humidity and temperature are major factors that can affect the physiology and thus the development, longevity and oviposition of many insects (Gullan and Cranston, 2005). Different exposures of relative humidity and temperature on stored product pests have been reported to show increased insect mortality as relative humidity decreased (Lale, 1992).

Conclusion

Based on the findings of the study; it is therefore, concluded that grain breakage affect/favour the development of *C. ferrugineus* while the whole grain prevents (hinder) the development of *C. ferrugineus*. To avoid the development of *C. ferrugineus* during storage, the stored grain should be free from breakage and hence all grains meant for storage should be wholesome. Also, relative humidity has significant effect on the population and emergence (development) of *C. ferrugineus* as different exposures to decrease in relative humidity had an increase effect on mortality of *C. ferrugineus*. Relative humidity can affect the physiology and thus the development, longevity and oviposition of *C. ferrugineus*. Therefore, it is advisable to store grains and keep our storage facilities in a condition not suitable for insect to manifest. Grains particularly maize should be stored at very low moisture content before storage and also proper aeration of the storage facilities or structures should be adhered to in order to keep insect infestation to the minimum in storage.

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