International Journal of Tropical Insect Science (2022) 42:3419–3426
https://doi.org/10.1007/s42690-022-00874-8

ORIGINAL RESEARCH ARTICLE

Assessment of Adzuki bean beetle (*Callosobruchus chinensis*) resistance in undamaged grains of bulk stored lentil

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Received: 5 October 2021 / Accepted: 10 August 2022 / Published online: 15 August 2022

Abstract
The Adzuki bean beetle (ABB), *Callosobruchus chinensis* infests and produces several successive generations in stored lentil (*Lens culinaris*); although all grains are not equally damaged by the insect. Thus, to answer the question why some of the grains are left undamaged, this study was conducted with the objective of determining if the observed difference either as damaged or undamaged in a stored grain is due to the difference in level of resistance to ABB. To achieve this objective a standard choice and no-choice tests were conducted using four lentil genotypes and 0 to 24 h old ABB. The two genotypes were derived from damaged lentil grains by ABB. Lentil genotypes and genotype by age interaction did not affect the number of eggs laid per female per day; the proportion of grains with eggs or holes; eggs per grain, holes per grain and mean developmental time. On the other hand, ABB age significantly affected all the parameters considered. The number of eggs laid per female per day increased from age two to age three and declined thereafter. All parameters but mean developmental time followed a similar patter as the number of eggs per female per day. On the contrary, mean developmental time increased continuously as the age of the ABB increased. This study confirmed that grains left undamaged in bulk stored grain are not resistant to Adzuki bean beetle.

Keywords Mean developmental time · Adult emergence · Egg number · Antixenosis · Egg distribution · Insect age

Introduction

The Adzuki bean beetle (ABB), *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae) infests stored lentil (*Lens culinaris* Medik.) grains in several lentil growing countries (Raina 1970; Kashiwaba et al. 2003; Tebkew and Mohammed 2006). For instance, in Ethiopia lentil grain infestation by this insect pest begins in the field and as a result of which grain damage and weight losses in lentil grains stored for 13 months ranges from 1.4 to 12.8% and 1.8 to 4.4%, respectively (Kidane and Habteyes 1989). Similarly, other report indicates that 10 to 58% of grains of landrace lentil (local) stored either in jute sack or clay pot have ABB egg and 0 to 6% them have adult emergence hole; whereas 20 to 63% of grains of the improved cultivar chalew (NEL-358) stored either in jute sack or barrel have ABB eggs and the proportions of grains with ABB adult emergence hole were 4 to 8% (DZARC 1991). Because Ethiopian farmers grow lentils from seeds that they stored from previous harvest, the ABB is one of the limiting factors that contribute to the low productivity and production of lentil. The consequences of grains damaged by ABB in subsistence type of farming system are two folds. First, insect infested grains lose weight, and/or become inconsumable, and have low market prices (Javaid and Poswal 1995). Second, insect infested grains have reduced germination capacity and vigor and these reductions directly commensurate with the number of emergence holes on a seed (Baier and Webster 1992). For instance, according to Ujagir and Byrne (2009) germination of large and small lentil varieties was 84 and 25%, respectively, when the seeds had one hole each; 32 and 5% when there were two holes per seed; and 20 and 0% when each seed had three holes.

Although Southgate (1979) states that bruchids are capable to produce multiple generations on the same seed until the food reserves are completely used up, in a stored grain all grains are not equally damaged by the bruchids. This phenomenon compels to raise a question "are undamaged

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grains in a bulk store resistant to a particular bruchid species?" Generally plant resistances to insects are categorized into three as antixenosis (non-preference), antibiosis and tolerance. Antixenosis (non-preference) is defined as a set of plant (grain in this case) traits and insect reactions that prevents usage of a given plant (in this case grain) as food (Radchenko and Tyryshkin 2004). Thus, it was speculated that grains that are left undamaged in a grain mass are non-preferred and thus are resistant to that particular insect pest. Besides, in some countries such as India, works have been done to screen lentil genotypes for ABB beetle resistance. For instance, Singh et al. (1980) and Gore et al. (2016) tested ABB preference for egg laying on lentil cultivars and wild *Lens* species along with other legume crops and found that the interspecific preferences were greater than the intraspecific preferences. The standard method of screening for resistance is artificially exposing known number of grains in a container to known number, age, and sex of a target storage insect pest. The major criteria for measuring resistance are ovipositional preference, adult emergence, percentage of damaged or weevil infested seed, insect developmental periods, and/or reproductive capacity of females exposed to seed of different cultivars (Clement et al. 1994). Through these screening methods resistant genotypes have been identified and resistant mechanisms have also been studied. However, if the grains left undamaged in stored grain are resistant type, then selection of such grains from damaged stored grains will be an additional screening method. Besides, for most pulse crops such as cowpea, chickpea, faba bean and field pea sources of resistance to ABB have been identified; whereas there are limited research works, particularly in Ethiopia, on lentil resistance to ABB. Therefore, the objective of this study was to determine if the observed difference either as damaged or undamaged in a stored grain is due to the difference in level of resistance to Adzuki bean beetle.

Materials and methods

Seeds of lentil genotypes ILL-7664 and ILL-2595, which are resistant to pea aphid (*Acyrthosiphon pisum* Harris), were separately stored in paper bags (about 2.5 kg each) and left for natural ABB infestation. After the seeds were heavily damaged by several generations for one year and the ABB stopped breeding, the seeds were poured into a bowl filled with water. Then, a Tea strainer was used to remove all the seeds that were floating, while the seeds that sank down were taken out from the water and spread onto a paper (A4 size) on a table in a laboratory. Those seeds that were selected from ILL-7664 and ILL-2595 were designated as ILL-7664ABB and ILL-2595ABB, respectively. After drying the seeds were sown at Chefe Donsa (alt. 2400 m a.s.l., 8° 57″ N and 39° 6″ E) along with their respective uninfested parent seeds on 18 August 2020 and harvested in January 2021.

The ABB used in this experiment was obtained from infested lentil seed from the Chickpea and Lentil Research Program of the Debre Zeit Center. Then the ABB were multiplied on a susceptible lentil cultivar Derash until sufficient number of beetles was available for infestation. To get a uniform age of about 0 to 24 h old ABB, the stock culture was sieved with a sieve; all adults were removed; and the ABB that emerged 24 h after sieving were used for the study. The experiment was conducted between 15 June 2021 and 30 August 2021in a laboratory at Debre Zeit Agricultural Research Center, Debre Zeit, Ethiopia. The room temperature at which the experiment was conducted is shown in Fig. 1. Moreover, the relative humidity within the experimental laboratory was estimated between 70 and 75% because in the area where Debre Zeit is located the main rainy season spans from June to September.

Choice experiment

For each genotype 30 seeds were placed in vial cap of 4 cm diameter and 1 cm deep. A total of 40 unsexed 0 to 24 h old ABB were placed in a petri dish (9 cm diameter and 1 cm deep). Then, the test arena was set by placing the petri dish at the middle and the caps with seeds around the petri dish in equidistant from each other. The arena was covered with plastic bowl to prevent the released ABB from escape. The beetles were allowed to lay eggs for five days because they lay more than 95% of their eggs within five days after emergence (data from no-choice experiment). After five egg laying days, the ABB were removed and the grains were transferred to vials. For each genotype there were six replications in completely randomized design.

No-choice experiment

A total of 30 seeds of each of the test genotypes was put into a vial (8.5 cm high, 4.5 and 3.5 cm bottom and top diameter, respectively) and infested with eight unsexed 0 to 24 h old ABB. The sex ratio in ABB is 1:1 (Bhattacharya and Banerjee 2000). Thus, it was assumed that four of the beetles were female and the other four were male. After the introduction of the ABB, the vial was closed with its cap, which was perforated to allow free air movement. The ABB were allowed to lay eggs for 24 h and then they were transferred to a fresh batch of the same genotype of lentil and the infested grain was kept for counting emerged adults. This was repeated each day until the entire ABB died. Each vial was labeled by the genotype name and the date of exposure to ABB. The experiment was arranged in completely randomized design with six replications.

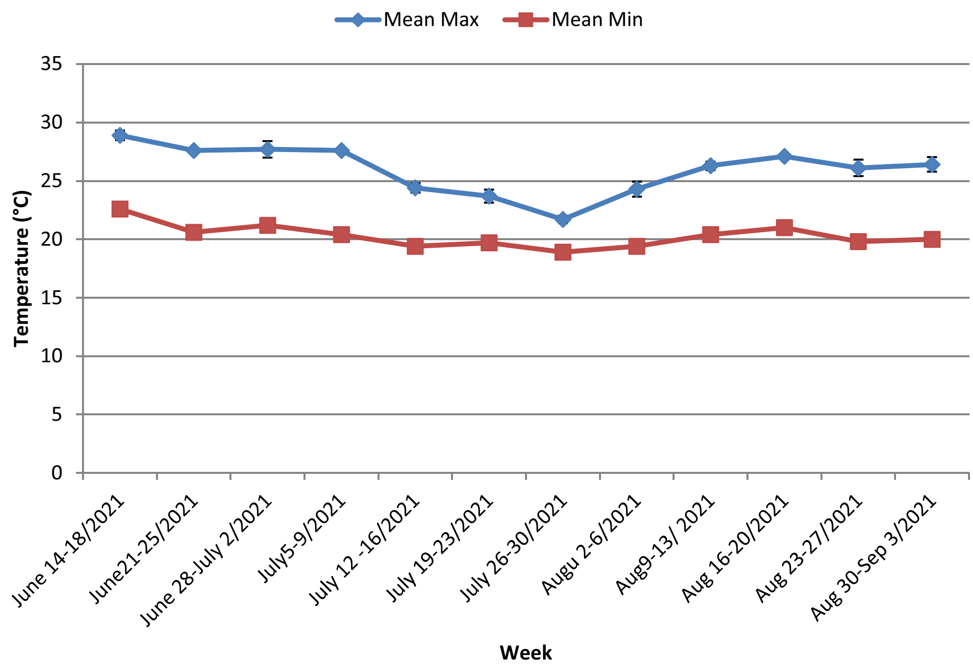

Fig. 1 Average daily room temperature (°C) at Debre Zeit laboratory during the experimental period. The bars indicate standard errors of means

Data collection

From each experimental set; each genotype; and infestation date data on number of eggs laid per grain, grains with and without eggs; adult emergence date, grains with and without hole; and number of holes from which adult emerged per grain were recorded. Eggs were counted five days after they were laid and they turned opaque as a result of debris from larval penetration of the seed. To record adults that emerged from grains, the vials were checked daily and the adults that emerged were continually counted and removed from the vial until no adult emerged from each replication. From these data the mean of total development period was calculated as, total mean development period = $(D_1A_1 + D_2A_2 + D_3A_3 + \ldots + D_iA_i)$ / Total number of adults emerged; where D_i- is the number of days from when the first adult emerged, A_i is number of adults emerged on D_i^{th} day. Total mean development period is the time taken from egg to adult emergence. The data from each experimental set was analyzed separately. Proc ANOVA of SAS (version 9.00) was used to compare the difference among genotypes in the proportion of grains with egg; the average number of eggs per grain; proportion of grains with holes; and the average number of holes per grain. However, before data analysis all the percentage data were transformed to arc sine scale but only the actual values were reported. To determine the effect of ABB age on different variables, the data from choice experiment were analyzed using repeated measure design (Proc mixed of SAS (version 9.00)) assuming compound symmetry covariate structure. Depending upon the result of the F-test, all statistical tests were carried out either at $p = 0.05$ or $p = 0.01$ level of significance.

Results

Choice test

There was no statistically significant ($p > 0.05$) difference among genotypes in the proportion of grains with egg; the average number of eggs per grain; proportion of grains with holes and the average number of holes per grain (Table 1). Thus, in all the tested genotypes more than 98% of the grains had egg on them and the average number of eggs per grain was greater than two. Similarly, more than three-fourth of the grains in each genotype had ABB emergence hole and the average number of holes per grain was one.

The ABB distributed their eggs on grains in similar pattern on all genotype (Fig. 2). Thus, depending upon the genotypes 38% to 48% of the grains had two eggs; 25 to 36% of the grains had three eggs; 7 to 12% had four eggs and 10 to 21% had one egg on them. The percentage of grain that were egg free (zero egg per grain) or had five eggs was at most 1.6%.

Similarly, the proportions of grains from which adult ABB emerged per grain were identical on all genotypes

Table 1 Adzuki bean beetle performance on different lentil genotypes

Genotypes	GE [a]* (%, n = 30)	ANEG [a]*	GH [a]* (%, n = 30)	ANHG [a]*	AE (%)
ILL-2595	98.39 ± 1.61	2.26 ± 0.18	76.37 ± 4.76	0.97 ± 0.08	42.92
ILL-2595ABB	98.89 ± 1.11	2.59 ± 0.09	88.37 ± 1.53	1.27 ± 0.07	49.03
ILL-7664	100.00 ± 0.00	2.35 ± 0.14	82.61 ± 2.81	1.15 ± 0.09	48.93
ILL-7664ABB	98.37 ± 1.11	2.28 ± 0.13	83.40 ± 1.83	1.13 ± 0.05	49.56

Abbreviations: GE, grains with egg; ANEG, average number of eggs per grain; GH, grains with hole; ANHG, average number of holes per grain; AE, adult emergence

[a] = mean ± standard error (SE); * = F-test non-significant at p = 0.05

(Fig. 3). Thus, one, two and three adult ABB per grain had emerged from 52 to 58%; 21 to 30% and 2 to 4% of the grains, respectively. However, there was no grain from which four or more adult ABB per grain were emerged in any of the tested genotypes. Besides, 12 to 24% of the grains had no adult emergence hole. Also there was no statistical difference ($p > 0.05$) among genotypes in the proportions of eggs that reached adult stage. Thus, nearly half of the eggs laid on grains of ILL-2595ABB, ILL-7664, and ILL-7664ABB reached adult stage. On the other hand, in grains of ILL-2595 adult ABB emerged only from 43% of the eggs (Table 1).

No-choice test

There was no statistically significant ($p > 0.05$) difference among genotypes and genotype by age interaction in the proportion of grains with eggs and average number of eggs per grain (Figs. 4 and 5). On the other hand, the proportion of grains with eggs and the average number of eggs per grain per day were significantly ($p < 0.01$) affected by age of ABB. Thus, the proportion of grains that had eggs on them (Fig. 4) and the average number of eggs per grain (Fig. 5) increased from age two to age three, but they decreased thereafter as the age of ABB increased. Among genotypes, although statistically non-significant, grains of ILL-2595ABB had the highest proportion of grains with eggs and also had the highest number of eggs per grain per day. On the other hand, the genotype ILL-7664 had the lowest proportion of grains with eggs and eggs per grain per day (Figs. 4 and 5).

The effects of genotype, ABB age and age by genotype interaction on the proportion of grains with hole and the average number of holes per grain were similar to the effect on proportion of grains with egg and average number eggs per grain (Table 2). Thus values of both parameters increased from age two to age three but declined continuously thereafter.

The mean number of eggs laid per female per day was unaffected by genotypes and genotype by ABB age

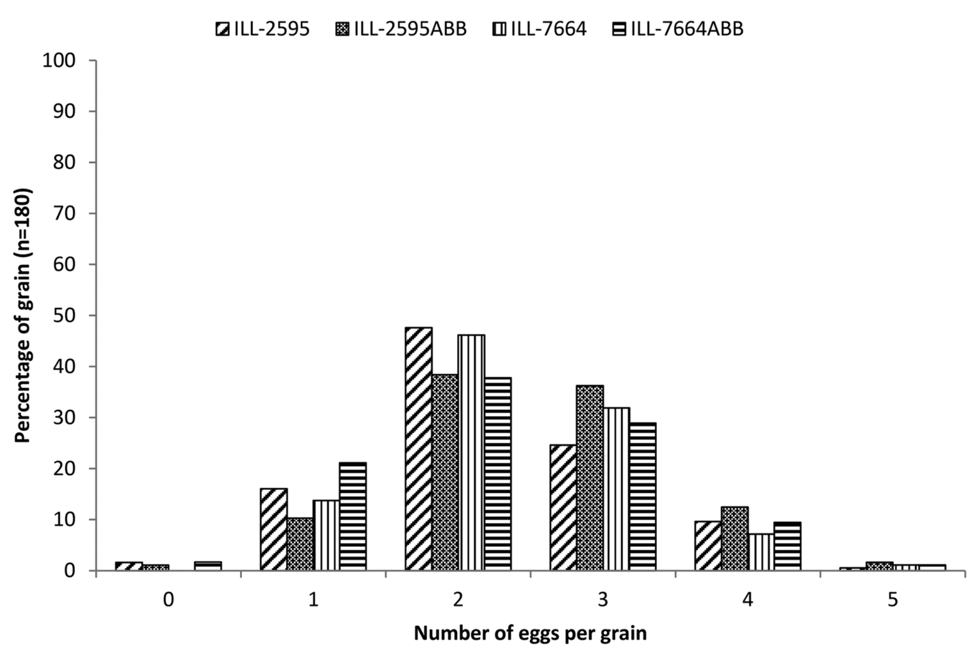

Fig. 2 Distribution of Adzuki bean beetle eggs per grain in choice test

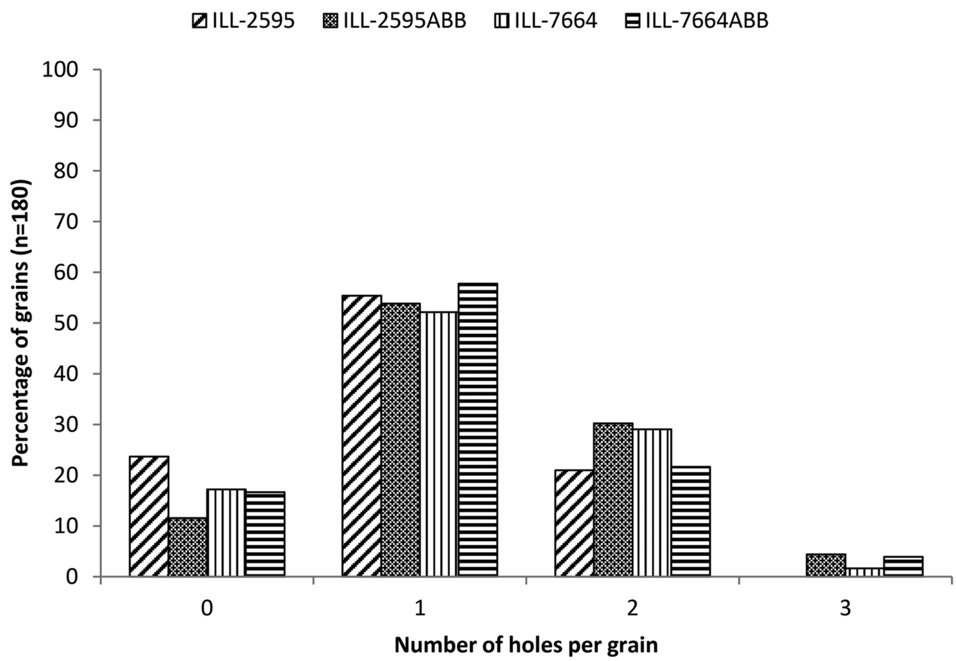

Fig. 3 Distribution of Adzuki bean beetle emergence hole per grain in choice test

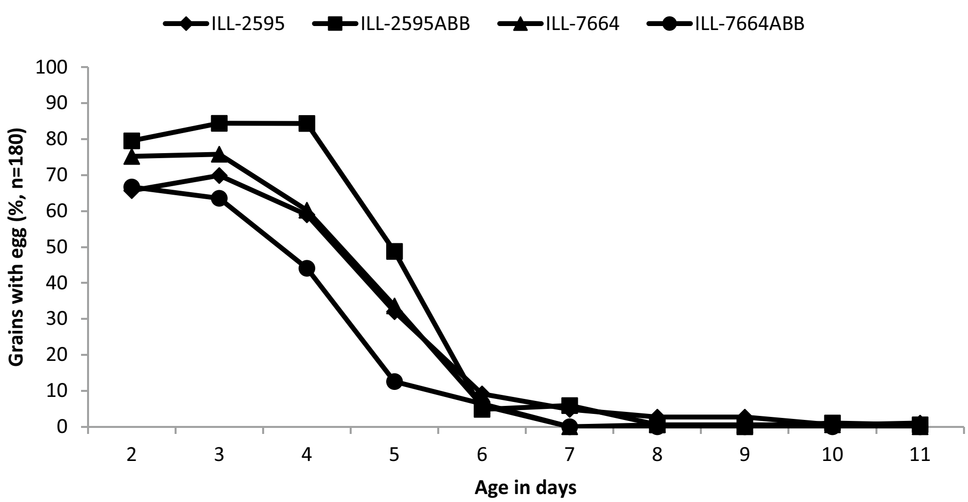

Fig. 4 The effect of Adzuki bean beetle on the proportion of grains with eggs in no-choice test

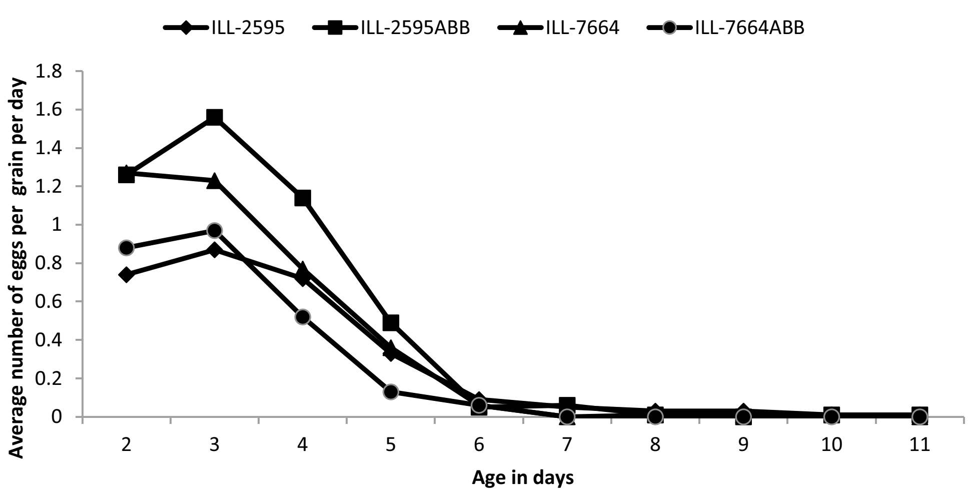

Fig.5 The effect of Adzuki bean beetle on the average number of eggs per grain in no-choice test

Table 2 Proportion of grains (n = 30 per replication) with holes and average number of holes per grain

Genotypes	Age in day	PGH [a*]	ANHG [a*]	Genotypes	Age in day	PGH [a*]	ANHG [a*]
ILL-2595	2	52.88 ± 1.44	0.59 ± 0.02	ILL-2595	5	18.00 ± 8.81	0.19 ± 0.09
ILL-2595ABB	2	62.37 ± 11.91	0.84 ± 0.18	ILL-2595ABB	5	30.75 ± 6.03	0.31 ± 0.06
ILL-7664	2	62.15 ± 11.89	0.97 ± 0.26	ILL-7664	5	16.10 ± 6.35	0.16 ± 0.06
ILL-7664ABB	2	54.07 ± 12.87	0.74 ± 0.11	ILL-7664ABB	5	6.56 ± 3.36	0.07 ± 0.04
ILL-2595	3	56.94 ± 12.02	0.67 ± 0.16	ILL-2595	6	2.69 ± 0.99	0.03 ± 0.01
ILL-2595ABB	3	75.23 ± 10.80	1.02 ± 0.19	ILL-2595ABB	6	3.77 ± 1.51	0.04 ± 0.02
ILL-7664	3	63.86 ± 15.59	0.93 ± 0.28	ILL-7664	6	1.61 ± 1.10	0.02 ± 0.01
ILL-7664ABB	3	54.34 ± 13.01	0.68 ± 0.18	ILL-7664ABB	6	3.33 ± 2.11	0.03 ± 0.02
ILL-2595	4	34.54 ± 9.45	0.37 ± 0.10	ILL-2595	7	4.35 ± 2.01	0.04 ± 0.02
ILL-2595ABB	4	58.62 ± 7.30	0.67 ± 0.10	ILL-2595ABB	7	4.30 ± 4.30	0.04 ± 0.04
ILL-7664	4	44.09 ± 13.65	0.49 ± 0.16	ILL-7664	7	0	0
ILL-7664ABB	4	24.83 ± 8.23	0.27 ± 0.09	ILL-7664ABB	7	0	0

Abbreviations: PGH, proportion of grains with hole; ANHG, average number of holes per grain

[a] = mean ± standard error (SE); * = F-test non-significant at p = 0.05 (among genotypes)

interaction. However, it was significantly ($p < 0.01$) affected by age of Adzuki bean beetle (Table 3). Thus, on all genotypes the number of eggs laid per female per day increased from age two to age three. But it decreased continuously beginning from age four until the ABB died. Generally, depending upon the type of lentil genotype, an ABB laid 22 to 35 eggs in her lifetime.

Genotypes and genotype by ABB interaction did not affect the average number of progenies per female (Table 4). However, age of ABB significantly affected the mean number of progeny per female. Thus, mean number of progeny increased from age two to age three, but it declined with increase in ABB age. In both experiments, the proportions of eggs that reached adult stage in grains ILL-7664ABB were generally greater than the proportion of eggs reached adult stage in the other genotypes (Table 4).

Mean development time (days) of ABB on lentil was not affected by genotypes, genotype by beetle age interaction (Table 5). However, the effect of ABB age on mean developmental time was significant and it increased as the age of the ABB increased. Depending upon the type of lentil genotype the mean developmental time of ABB ranged from 36 to 42 days (Table 5).

Table 3 Eggs per female Adzuki bean beetle per day laid on lentil grain

Genotypes	Age in days [a*]										Total
	2	3	4	5	6	7	8	9	10	11	
ILL-2595	6.57 ± 0.55	7.68 ± 1.90	6.28 ± 1.59	2.79 ± 0.86	0.83 ± 0.35	0.42 ± 0.17	0.21 ± 0.14	0.21 ± 0.21	0.04 ± 0.04	0.08 ± 0.05	25.11
ILL-2595ABB	8.13 ± 1.94	10.16 ± 2.45	7.16 ± 1.03	3.21 ± 0.45	0.31 ± 0.16	0.46 ± 0.46	0.04 ± 0.04	0.00 ± 0.00	0.08 ± 0.05	0.03 ± 0.03	29.58
ILL-7664	12.68 ± 3.22	11.39 ± 3.50	6.89 ± 2.03	3.22 ± 0.91	0.50 ± 0.23	0.00 ± 0.00	0.04 ± 0.04	0.08 ± 0.08	0.06 ± 0.06	0.00 ± 0.00	34.86
ILL-7664ABB	7.47 ± 2.11	8.07 ± 2.11	4.49 ± 1.41	1.27 ± 0.60	1.14 ± 0.98	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22.44
Mean (for age)	8.71	9.32	6.20	2.62	0.69	0.22	0.07	0.07	0.04	0.02	

[a] = mean ± standard error (SE); * = F-test non-significant at p = 0.05 (among genotypes)

Table 4 Average number of progenies per female Adzuki bean beetle per day

Genotype	Age in days [a*]						Total	Adult emergence (%)
	2	3	4	5	6	7		
ILL-2595	5.13 ± 0.30	6.74 ± 0.95	3.18 ± 0.88	1.50 ± 0.69	0.25 ± 0.10	0.38 ± 0.18	17.18	68.42
ILL-2595ABB	5.70 ± 1.35	6.59 ± 1.43	4.33 ± 0.67	2.03 ± 0.41	0.24 ± 0.09	0.33 ± 0.33	19.22	64.98
ILL-7664	9.07 ± 2.55	8.75 ± 2.83	4.24 ± 1.26	1.29 ± 0.53	0.21 ± 0.14	0.00 ± 0.00	23.56	67.58
ILL-7664ABB	6.59 ± 1.19	6.75 ± 0.97	2.75 ± 0.86	0.68 ± 0.27	0.74 ± 0.65	0.00 ± 0.00	17.51	78.03

[a] = mean ± standard error (SE); * = F-test non-significant at p = 0.05 (among genotypes)

Table 5 Total developmental time (in days) of Adzuki bean beetle on lentil

Genotypes	Age in days [a]*					
	2	3	4	5	6	7
ILL-2595	36.44 ± 0.29	37.11 ± 0.18	39.13 ± 0.71	38.14 ± 0.84	42.38 ± 1.77	41.44 ± 1.75
ILL-2595ABB	36.88 ± 0.30	37.20 ± 0.34	38.23 ± 0.42	39.23 ± 0.42	42.75 ± 1.03	37.12 ± 10.99
ILL-7664	36.15 ± 0.45	36.10 ± 0.63	37.20 ± 0.33	37.68 ± 0.29	39.92 ± 2.58	-
ILL-7664ABB	35.69 ± 0.32	36.35 ± 0.26	37.49 ± 0.45	38.31 ± 0.67	42.33 ± 1.33	-
mean (for age)	36.29	36.69	38.01	38.34	41.85	39.28

[a] = mean ± standard error (SE); * = F-test non-significant at $p = 0.05$

Discussion

There were no statistically significant differences among genotypes in any of the variables considered both in choice and no-choice experiment. Thus, in both experiments the ABB laid more than 95% of their eggs on all genotypes in the first five days of their life time. This result corroborates with report by Raina (1971) who reported that the species *C. chinensis*, *C. maculatus* and *C. analis* lay 95% of their eggs in the first five days of their life. The total number of eggs laid by an individual ABB in this experiment is within the range of eggs found by Bhattacharya and Banerjee (2000, 2001), who reported an average number of about 3 to 86 eggs per female.

In this study, the percentage of adults emerged from both choice and no-choice were less than those reported on lentil by Singh et al. (1980), who reported 95% adult emergence; while it was comparable with the adult emergence found by Bhattacharya and Banerjee (2000, 2001). According to Bhattacharya and Banerjee (2001), lentil seed-coat contains high amount of phenols that reduce the penetration capacity of ABB neonate larvae. Thus, it is believed that the phenol content of Ethiopian lentil seed-coat is similar to those reported by these authors. Consequently, most ABB neonate larvae might have not able to penetrate the seed-coat of the tested lentil genotypes. The inability of neonate larvae to penetrate seed-coat in turn reduces the proportion of eggs that reaches adult stage.

The developmental time from egg to adult was about seven weeks and increased as the age of the ABB was increased. This developmental time is greater by two weeks than the developmental time reported by Gore et al. (2016), but it is comparable to the developmental time of ABB reared on azuki bean (*Vigna angularis* [Willd.] Ohwi & Ohashi) at 22 °C (Maharjan et al. 2017). According to Fox (1993) in *C. maculatus* as age of female increases, the proportion of eggs that hatch and the larval survival rate decrease; on the other hand, the developmental time increases. This author attributed this effect to the nutritional status of the females and her egg contents, which decreases as age increases.

The standard error of means for most variables such as the proportion of grains with eggs or holes; average number of eggs per female per day were larger relative to their respective means. This indicates that the variability among replications of a genotype was high, which might be partly attributed to the difference in ABB sex ratio used for the experiment. It is known that the sex ratio in ABB is 1:1 (Bhattacharya and Banerjee 2000). However, in this study out of the total 24 experimental units 46% of them had male to female ratio of 1:1; 29% had more males than females and the remaining 25% had greater number of females than males. On the other hand, the use of unsexed storage insects is common in storage experiments. For instance, Keneni et al. (2011a, b) assessed chickpea resistance to ABB using unsexed adults and found location by genotype interaction in the number of eggs per female; number of adults emerged (first progeny); and adult recovery, although these responses are expected to be static (Becker and Leon 1988). Similarly, Kim and Kossou (2003) used unsexed maize weevil (*Sitophilus zeamais* Motschulsky) to assess maize resistance to this weevil. However, according to Miyatake and Matsumura (2004) in cowpea weevil (*C. maculatus*) unpaired males disrupt mating pairs in conditions where there are more males than females, which in turn affect the performance of the females. Thus, it is recommended either to increase the number of replications if experimental insects are not sexed and discard those experimental units with sex biases or use only sexed individuals.

Conclusion

The statistically non-significant differences among genotypes in any of the variables considered confirm that the genotypes ILL-2595ABB and ILL-7664ABB had no grain characteristics that hinder egg laying by the ABB or grain content that affects ABB development. Had this been true, grains of these genotypes would have the lowest proportion of grains with eggs or holes; the lowest number of eggs per grain or holes per grain; and the longest total mean developmental time. Moreover, in terms of insect performance, the number of progenies per female is expected to be less than the number of progenies from their counter parts. Thus, this study confirms that grains left undamaged in bulk stored lentil are not resistant to Adzuki bean beetle.

Acknowledgements I acknowledge Mr Ashebir Tolera for his help during data collection and the Ethiopian Institute of Agricultural Research for financial support. Also acknowledgement is due to anonymous reviewers who critically reviewed and improved the manuscript.

Declarations

Conflict of interest I declare that there is no conflict of interest in this work.

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