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Elimination of the Common Bean Weevil *Acanthoscelides obtectus* (Say) by Hermetic Storage of Dry Common Bean at Different Moisture Contents

Marco Antonio GARCÍA-PEREA[1] Sergio JIMÉNEZ-AMBRÍZ[1]
Fernando CASTILLO-GONZÁLEZ[2] Abraham MÉNDEZ-ALBORES[1]
Ernesto MORENO-MARTÍNEZ[1*]

[1] UNAM-FESC, UNIGRAS. Av. Jorge Jiménez Cantú S/N. Cuautitlán Izcalli. C.P. 57740, Estado de MÉXICO
[2] Colegio de Posgraduados. Campus Montecillo. Posgrado en Recursos Genéticos y Productividad-Genética. Km 36.5 Carretera México-Texcoco. C.P. 56230, Estado de MÉXICO
e-mails: [1*]ernesto@unam.mx, markovan31@hotmail.com, [1]sjimenez@unam.mx, [2]fcastill@colpos.mx, [1]albores@unam.mx

ABSTRACT

The objective of this research was to determine the required storage time as well as the dry common bean (*Phaseolus vulgaris* L., Fabaceae) moisture content, to favor oxygen depletion and carbon dioxide increment for elimination of all stadia of the common bean weevil *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Experimental units with 150 g of dry bean (with moisture content around 10, 12, and 16%) infested with 20 adult insects were stored 21 d under hermetic and non-hermetic conditions. Oxygen/carbon dioxide levels, moisture content, insect mortality of initial infestation, emergence of the insects from the first generation (F_1) and percentage of damaged dry bean were registered. In non-hermetic storage, the dry bean presented up to 395 insects from F_1; and with damage up to 51.1%. Oxygen and carbon dioxide levels which did not allow the development (F_1 emergence) of *A. obtectus* and the subsequent damage to the dry bean were between 0.5 - 3.5% and 8.9 - 9.7%, respectively. Hermetic storage system after 6, 9 and 12 d did not showed damage for moisture contents of 10, 12 and 16% respectively. With low moisture contents of the grain, there is lower consumption of oxygen and less carbon dioxide production by adult insects. On the contrary, with high grain moisture content insect activity increases with more consumption of oxygen and more carbon dioxide in less time, these results showed that high carbon dioxide and low oxygen levels joined with low grain moisture inhibit insect development in less time under hermetic storage.

Key words: Acanthoscelides obtectus (Say), dry common bean, hermetic storage.

INTRODUCTION

Food availability for the rural populations of Latin America depends greatly on the capacity of farmers to preserve the quality of the maize produced (*Zea mays* L., Poaceae) and dry common bean (*Phaseolus vulgaris* L., Fabaceae). In many geographical areas, a negative factor in food preservation has always been the deficiencies in grain postharvest systems and lack of technical assistance. Farmers usually keep their produced grains inside their own living quarters with great quantitative and qualitative losses caused mainly by insects. In the case of dry common bean, the two major insect pests are the common bean weevil *Acanthoscelides obtectus* (Say) and the Mexican bean weevil *Zabrotes subfasciatus* (Boheman) both (Coleoptera: Bruchidae) (Gatehouse *et al*., 1998; López-Pérez *et al*., 2007). The losses caused by these two insects have dramatic consequences for small farmers, either in temperate zones where *A. obtectus* thrives or on warm zones due to the presence of *Z. subfasciatus*; since the common dry beans represent their daily food supply, as well as the annual product of their work effort.

Hermetic storage of grains is well-recognized as an effective method to reduce the damage caused by insects during storage (Navarro and Donahaye, 1990; Navarro *et al*., 1993). The success of hermetic storage is based on the exhaustion of oxygen and generation of carbon dioxide in the grain atmosphere container (Varnava *et al*., 1995). The use of dangerous insecticidal applications and expensive fumigations require resources and knowledge bases that are not generally available to farmers in rural areas of underdeveloped regions. Therefore, assistance efforts should be focused on providing an ecological and user-friendly storage system, such as hermetic grain storage (Porca *et al*., 2003).

An investigation of hermetically-stored maize revealed that respiration of insects is the main cause of oxygen consumption in the storage atmosphere, followed by fungi and finally by the maize grains themselves (Moreno *et al*., 2000). These findings are not only of academic interest, but also of practical use for the development of lethal atmospheric methods to control the proliferation of storage insects and fungi. Consequently, in order to eliminate or minimize the damage caused by insects in rural settings of dry common bean storage, without the use of pesticides, more information is needed including an understanding of the physiological behavior of insects to guide the proper use of hermetic storage. Using the techniques of hermetic storage, this research was carried out to determine the levels of oxygen and carbon dioxide in function with the moisture content required for the complete elimination of eggs, larvae and pupae of the insect *A. obtectus*, without the use of insecticides.

MATERIALS AND METHODS

Dry common bean

Dry common bean, (*P. vulgaris* L.), of the Mayocoba variety, produced in Sinaloa, Mexico, was used for this research. At the point of arrival at the laboratory, the dry common bean presented a moisture content of 10.7%, grain germination 96% and a slight invasion (2%) by the fungus *Alternaria* spp. The bean was placed for 10 d at −4°C to eliminate any possible pre-or postharvest insect infestation with eggs, larvae, pupae or adults (Teixeira and Zucoloto, 2003).

Insect population

A population of the weevil *A. obtectus* from the entomology laboratory of UNIGRAS was reared on dry common bean (*P. vulgaris* L.) of the Mayocoba variety (free of pesticides) in a chamber at 28±2ºC, and 75±5% relative humidity with a photoperiod of 12 h light. The weevils were reared in 10 glass jars of one gallon with 1.5 kg of dry beans and 500 weevils without sexing. After 5 d the insects were removed. Insects that emerged during the first four days were eliminated and the next day the emerged insects were used in the experiment with less than 24 h of emergence.

Moisture content

The moisture content of the dry bean was determined by an oven-drying method according to the rules of the International Seed Testing Association (ISTA, 1996). The moisture content percentage was calculated on the basis of the wet weight of the bean. The moisture content in each sampling was immediately determined subsequent to the oxygen and carbon dioxide measurements.

Oxygen and carbon dioxide determination

The percentages of oxygen and carbon dioxide were determined by using digital gas analyzer equipment Illinois^{TM}, model 6600 (Illinois Instruments Inc., Johnsburg, Illinois, IL, USA). The instrument is automatically self-calibrating, based on the normal amount of air from the environment, with oxygen contents at 20.9% and carbon dioxide at 0.03%. In 15 sec, the gas analyzer takes a sample of 38 mL of air. The empty air-tight flasks have a 275 mL capacity; the 150 g of dry beans occupied 110 mL, leaving head space and inter-granular air of approximately 165 mL [enough for only one storage atmosphere sampling performed in each experimental unit]. The oxygen and carbon dioxide contents were the first variables determined in each one of the three replicates for each sampling period.

Infestation and storage

A. obtectus infestation and storage were achieved as follows: from a lot of dry common bean, three sub lots of 6.3 kg were each adjusted to moisture content of 10, 12, and 16%, by exposing the dry common bean under three different relative humidities maintained by means of saturated salt solutions. For 10% moisture content, the salt used was potassium carbonate; for 12%, sodium bromide; and for 16%, potassium chloride (Winston and Bates, 1960). Once the desired moisture content was achieved for each sub lot, each was divided into 42 experimental units of 150 g each. The experimental units were infested with 20 adult insects without sexing, and with less than 24 h post-emergence.

Twenty-one experimental units were placed in bottles which were hermetically sealed with metal covers, and a rubber disc was encrusted in the cover for the sampling of the storage atmosphere. The rest of the experimental units (21 of each sublot of moisture content tested) were used for the open storage system; the bottles were covered with wire mesh to prevent the escape of insects. The total number of the experimental units was 126 for the two storage systems, three moisture contents, and three replicates per each of the seven samplings.

All experimental units were placed in a rearing room at 28±2ºC, 75±5% relative humidity with a photoperiod of 12 h light; seven samplings were carried out every

3 d. In each one of the samplings, oxygen and carbon dioxide concentrations were determined along with moisture content as well as the percentage of dead and alive insects of initial infestation, which were retired from each experimental unit. Those experimental units which were already insects free were covered with wire mesh and then placed back in the rearing room for 50 d to allow for the emergence of the insects from the first generation (F_1) of the eggs laid by the initial insect population, and also for quantifying the percentage of damaged dry bean. Damage was quantified by the presence of exit insect holes and/or circular windows prepared by the last larval instar (Bhadriraju and Hagstrum, 1996), taking into account the total number of dry beans remaining in the experimental unit.

Experimental design and statistical analysis

The experiment was carried out under a completely randomized design. After testing normal distribution of data with the procedure "Univariate Normal" with the Anderson-Darling test and homogeneity of variance test of Bartlett, these tests showed that the data did not result from a normal distribution and with an absence of homogeneity of variances. Therefore a non-parametric test of Kruskal-Wallis using the procedure "npar1way" with the Wilcoxon test was performed (Zar, 1999). The comparison of means tests was carried out with ranked data utilizing Tukey test ($\alpha=0.05$) with the Statistical Analysis System (SAS, 2002). The tables show the average of three replicates from the original data, with the mean comparison of ranked data.

RESULTS

The statistical analysis showed highly significant differences ($P<0.01$) between open and hermetic systems throughout the 21 d storage period, as well between moisture contents oxygen, carbon dioxide levels, F_1 insect emergence, and grain damage; except for insect mortality at 15, 18 and 21 d, where there was no-significant difference ($P>0.05$).

For the hermetic storage system, from 3 to 21 d of storage, the dry bean moisture content averages were: for the desired 10% was 10.4%; for 12%, was 12.3%; and for 16%, was 16.4%. In the case of the open storage system, for the desired moisture contents of 10, 12, and 16%, the averages were: 10.6, 12.4, and 16.5%, respectively.

In the hermetic storage system, oxygen levels drastically decreased during the course of the brief storage period (Fig. 1). At only 3 d, the atmosphere of bean stored at 10, 12, and 16% moisture content had average oxygen contents of 10.8%; this data represents a reduction of around 50% of the original oxygen level (20.9%). At 6 d of storage, the oxygen levels were 3.5, 5.4, and 3.6%, respectively; and at the end of the storage period (21 d), the oxygen dropped to levels of 2.2, 1.8, and 0.5%, respectively. In relation to carbon dioxide, the atmosphere of the hermetic system presented at 3 d levels around 5% for all three moisture contents (10, 12, and 16%). After 6 d, carbon dioxide levels ranged from 8.1 to 9.7% (data not shown). As expected, the open storage system did not show any variation in relation to the levels of oxygen and carbon dioxide.

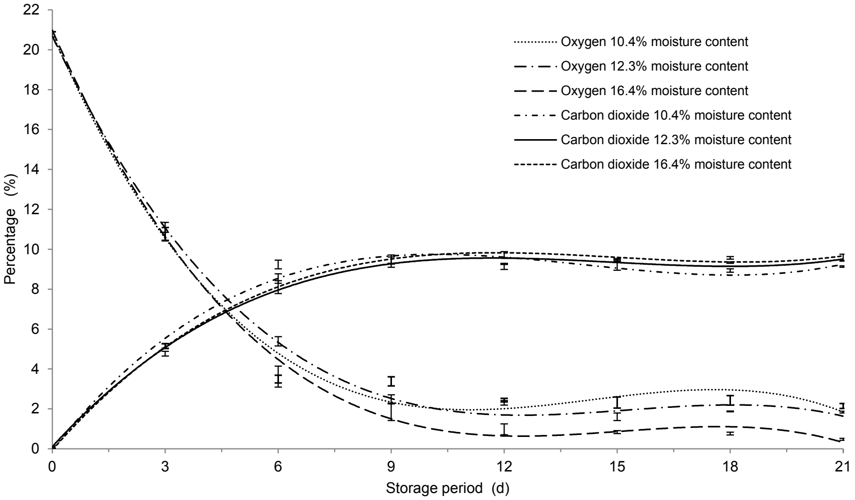

Fig. 1. Effect of the dry common bean moisture content on the environmental oxygen consumed and increased carbon dioxide by *A. obtectus*, fitted with a polynomial function.

Dry bean hermetically stored at 3 d presented with an insect mortality of 2, 3, and 7%; and at 6 d mortality of 80, 32, and 23%, for bean stored with moisture contents of 10, 12, and 16%, respectively. However, at 9 d, insect mortality was 100%, in all moisture contents tested (Table 1). Under non-hermetic storage conditions, at 3 d, for dry bean stored with 10% moisture content, the mortality rate was 2%, and for 12 and 16% moisture content insect mortality was 0%. At 6 d of storage, the insect mortality was 13, 12, and 10% for dry bean with 10, 12, and 16% moisture content, respectively. After 9 d the insect mortality increased dramatically from 48 to 100% (Table 1). It is interesting to note that in moisture contents of 10, 12 and 16% insect mortality occurred around 15 d, coinciding with the insect's life cycle (Bushnell and Boughton, 1940).

The number of emerged insects from F_1 in the hermetic storage system, for all moisture contents, at 3 d of storage was 111, 111, and 135 insects for bean with 10, 12, and 16% moisture content, respectively (Table 2). At 6 d, and thereafter there were 0 emerging insects in bean stored with 10% moisture content; however there were 38 and 24 emerging insects at 12 and 16% moisture content, respectively. After, 9 d for all moisture contents the insect emergence was 0, except for bean at 16% moisture content, with an emergence of 7 insects (Table 2). On the contrary, in the open storage system, insect emergence reached high numbers of insects. At the end of the storage period (21 d), insect emergence reached 284, 339, and 350 insects for dry bean stored with 10, 12, and 16% moisture content, respectively. However, for some samplings, the insect emergence was higher than those numbers given above, especially for the dry bean stored 15 d with 12% moisture content, reaching 395 insects (Table 2).

Table 1. Mortality of initial infestation of *A. obtectus* in stored beans with three moisture contents during 21 days.

		Storage period (d)						
System	MC (%)	3	6	9	12	15	18	21
Hermetic	10.4	2±0.3 c^{2}	80±2.9 ab^{1}	100±0.0 a^{1}	100±0 a^{1}	100±0 a^{1}	100±0 a^{1}	100±0 a^{1}
	12.3	3±0.3 c^{1}	32±1.7 b^{1,2}	100±0.0 a^{1}	100±0 a^{1}	100±0 a^{1}	100±0 a^{1}	100±0 a^{1}
	16.4	7±1.7 c^{1}	23±1.7 b^{2,3}	100±0.0 a^{1}	100±0 a^{1}	100±0 a^{1}	100±0 a^{1}	100±0 a^{1}
Non-hermetic	10.6	2±0.3 c^{2}	13±1.7 bc^{3,4}	63±1.7 b^{2}	98±1.7 a^{1}	100±0 a^{1}	100±0 a^{1}	100±0 a^{1}
	12.4	0 c^{3}	12±1.7 bc^{4}	55±2.9 b^{3}	97±3.3 a^{1,2}	100±0 a^{1}	100±0 a^{1}	100±0 a^{1}
	16.5	0 d^{3}	10±2.9 cd^{4}	48±1.7 bc^{3}	83±3.3 b^{2}	98±1.7 a^{1}	100±0 a^{1}	100±0 a^{1}

Original means of three replicates ± standard error.

Means with same letter (from ranked data) in the same row and means with same number in superscript (from ranked data) in same column are not significantly different (Tukey P > 0.05).

MC: moisture content.

Table 2. Emergence of *A. obtectus* from the first generation in stored beans with three moisture contents during 21 days

		Storage period (d)						
System	MC (%)	3	6	9	12	15	18	21
Hermetic	10.4	111±4.6 a^{3}	0 b^{6}	0 b^{3}	0 b^{2}	0 b^{4}	0 b^{4}	0 b^{2}
	12.3	111±5.5 a^{3}	38±3.2 b^{4}	0 c^{3}	0 c^{2}	0 c^{4}	0 c^{4}	0 c^{2}
	16.4	135±5.1 a^{1,2}	24±3.9 b^{5}	7±1.2 c^{2,3}	0 d^{2}	0 d^{4}	0 d^{4}	0 d^{2}
Non-Hermetic	10.6	158±5.1 bc^{1}	123±12.3 c^{3}	272±10.8 ab^{1}	249±14.3 abc^{1}	255±34.4 ab^{3}	298±13.6 a^{3}	284±52.5ab^{1}
	12.4	170±16.0 e^{1}	219±9.2 de^{2}	259±12.3 cd^{1,2}	298±13.9 bc^{1}	395±21.4 a^{1}	327±6.5 ab^{2}	339±14.6 ab^{1}
	16.5	127±4.4 d^{2,3}	260±5.0 bcd^{1}	251±12.4 cd^{1,2}	278±15.0 bc^{1}	310±3.5 ab^{2}	370±10.8 a^{1}	350±14.5 a^{1}

Original means of three replicates ± standard error.

Means with same letter (from ranked data) in the same row and means with same number in superscript (from ranked data) in same column are not significantly different (Tukey P > 0.05).

MC: moisture content.

Regarding the dry bean damage at 10% moisture content and 3 d, there was 17.6% damage, but thereafter there was no more damage; dry bean at 12 and 16% moisture content, after 12 d did not presented any damage (Fig. 2). The non- hermetic storage system, at 21 d presented 38.4, 47.1, and 49.0% for dry bean stored at 10, 12, and 16% moisture content, respectively (data not shown). The percentage of damaged grain stored under hermetic and open systems is highly correlated with the number of insects emerged in the three moisture contents evaluated in this research. The correlation coefficients (r) in hermetic system were 0.99 for all moisture contents, in non-hermetic system r values were 0.83, 0.95 and 0.96 for 10, 12 and 16% moisture content, respectively.

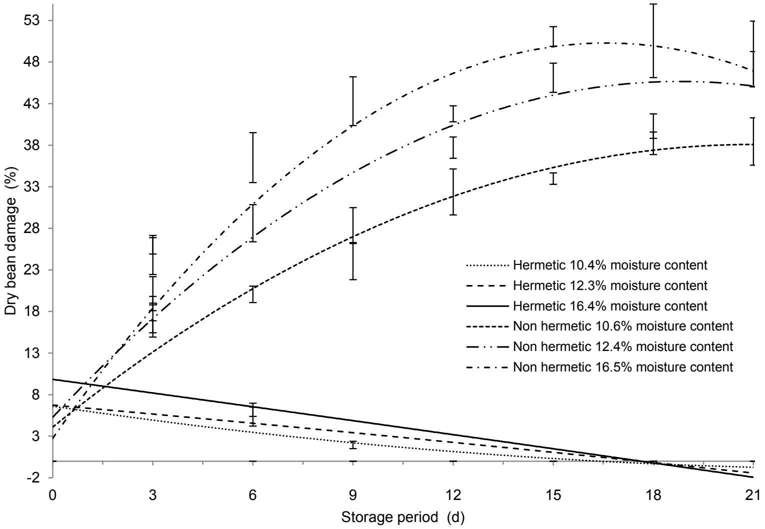

Fig. 2. Effect of moisture content and storage system on dry common bean damage fitted with a polynomial function.

DISCUSSION

The adjusted moisture content of the experimental unites were quite similar to the desired conditions. This correlates with the shorter storage period and rapid oxygen depletion. Literature pertaining to the effect of low oxygen pressure states that insects will perish if the oxygen level of the hermetic storage atmosphere should fall around 2% (Oxley and Wickenden, 1963). Also, Moreno *et al*. (2000), working with *Sitophilus zeamais* Motschulsky on hermetically stored maize, found at 9 d that the oxygen content was 1%, with 100% insect mortality, including eggs larvae and pupae. The results of this research are in close alignment with the above cited authors since the lethal oxygen levels of dry bean atmospheric conditions ranged from 0.5 - 3.5**%**, and for carbon dioxide were 8.9 - 9.7%, with non F_1 insect emergence and 0% of dry bean damaged (Figs. 1, 2; Table 2). The insect mortality of initial infestation started at 3 d with low rates, reaching at 9 d a 100% mortality with oxygen levels of 1.8 - 3.4%, and 9.2 - 9.5% carbon dioxide, respectively (Fig. 1; Table 1). In regard to carbon dioxide, Cheng *et al.* (2012) found that in atmospheres under low oxygen levels, carbon dioxide enhanced the effects of hypoxia on mortality, development and gene expression in cowpea bruchids. Emekci *et al*. (2002) pointed out the crucial need to generate precise information pertaining to what is happening with the physiology of the insects under such special environmental conditions of low oxygen. Different responses in relation to insect mortality, under different low oxygen pressures and under different moisture content, had been observed. *Prostephanus truncatus* Horn, at 6 days, presented 100% mortality in maize hermetically stored with 14, 15 and

17% moisture content (Quezada-Viay *et al*., 2006). On the other hand, adult insects of *Sitophillus. zeamais,* hermetically stored with a 15% moisture content at 12 days presented 100% mortality (Moreno *et al*., 2000)*, and Zabrotes. subfasciatus stored at* 10, 12 and 15% moisture content, presented a 100% insect mortality at 9 days in the three moisture contents tested, (unpublished data).

In relation to insect emergence (F_1) and dry bean damage, in bean stored 6 d with 12 and 16% moisture content, these conditions appeared to have a beneficial effect on egg eclosion, as well as on entrance and development of larvae and adults insects in the dry common bean, which it was observed by quantifying the number of emerging insects and the percentage of dry bean damaged in the rearing chamber. The same phenomenon was observed with the dry bean stored up to 9 d with 16% moisture content (Table 2 and Fig. 2). Thereafter, as can be seen in that table and figure, there was no insect emergence nor grain damage. The results herein described are due to the fact that these moisture contents (12 and 16%) are in equilibrium with a relative humidity at around 60 - 75%. These conditions allow the insect egg eclosion and development of this weevil (Papachristos and Stamopoulos, 2004). The dry common bean damage shown in these results is the direct response of insect egg hatching, larvae feeding and adult emergence. It is important to point out, under the circumstances of this research, that at only 3 d of non-hermetic storage (normal system of storage), under the three moisture contents tested (10, 12, and 16%), the dry bean damage was quite high around 22% (Fig. 2); since 10 and 12% moisture content are not considered to be high moisture content, and yet are commonly found in recently harvested dry bean.

In the open storage system, the average of emerging insects from F_1 was higher (Table 2), causing great physical damage up to 51.1% (Fig. 2); which is a problem that requires an urgent solution, mainly in underdeveloped regions, where even grains with low moisture content results in severe insect postharvest losses, which in turn creates a direct problem for access to sound grains, such as dry beans for auto consumption in marginal urban and rural areas.

This research clearly shows that the moisture content of the bean plays an important role in the development and longevity of the insects, since low moisture content such as 10 and 12%, promoted in fewer days of storage an earlier insect death than 16% did; however in the tree moisture contents no visible damage was observed through the storage period. This agree with Calderon and Navarro (1980) and Oxley and Wickenden, (1963), where they mention that the low moisture content in grains causes a dehydrating effect on the immature stages of insects, since insects need moisture to complete their life processes. With these results, it can be concluded that the three factors involved in creating a lethal storage atmosphere for the survival and development of *A. obtectus* are low oxygen levels, moderate high levels of carbon dioxide and low grain moisture content, synergetic factors to control grain storage insects.

Regarding the results of the hermetic storage of common dry bean can be summarized as follows: the lethal levels of environmental gases that did not allow the development of *A. obtectus* and prevented the subsequent damage to the stored dry bean ranged between 0.5 to 3.5%, for oxygen, and 8.9 to 9.7%, for carbon dioxide; and the safe hermetic storage periods were 6, 9 and 12 d for grain moisture contents of 10, 12 and 16%, respectively. The low moisture content was more adequate for dry common bean preservation.

Finally, for the optimal use of hermetic storage in rural areas for small grain volumes to eliminate eggs, larvae and adult insects (before reaching a 0% damage), this cannot be achieved by depending solely upon insect respiration; since the initial insect field infestation is not as large as the number of insects used in this research (twenty insects in only 150 g of maize). However, with the large volumes of commercial grain under hermetic storage conditions currently in many countries, the final losses will be very small compared to those inflicted during the normal open storage. Therefore, the commercially available modern hermetic storage is a very good alternative to preserving large amounts of grain (as in the case of maize) so greatly needed for human consumption.

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