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The Role of Moisture Content on the Preservation of Hermetically-Stored Dry Bean Infested with the Mexican Bean Weevil *Zabrotes subfasciatus* (Boh.)

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ABSTRACT

The aim of this work was to determine the effect of moisture content on dry common bean (*Phaseolus vulgaris* L., Fabaceae) during the insect disinfestation by oxygen depletion and carbon dioxide increment for elimination of all stages of the Mexican bean weevil *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) and avoidance of physical damage of dry bean occasioned by insect development. Experimental units with 150 g of dry bean with 10, 12, and 15% of moisture content infested with 20 adult insects sexing 1:1 were stored 27 d under hermetic and non-hermetic conditions. Oxygen and carbon dioxide levels, moisture content, and insect mortality of initial infestation were evaluated every 3 d. To quantify emergence of the insects from the offspring (F_1) and percentage of damage to dry bean, the dry bean of the experimental units free of adult insects was kept 50 d in a rearing room. Non-hermetically stored grain presented with insect emergence up to 475 insects and 53.7% physical damage. However, in the hermetic storage system, after 21 and 27 d, there was no insect emergence and no dry bean was damaged with moisture contents of 10 and 12%, respectively. Oxygen levels which did not allow the development (F_1) of *Z. subfasciatus* and the subsequent damage to the dry bean were between 3.6-6.0% and for carbon dioxide were 7.70-10.27%. These results show the importance of moisture content in dry bean to inhibit insect development of *Z. subfasciatus* in short time under hermetic storage conditions of low oxygen and high carbon dioxide levels.

Key words: Zabrotes subfasciatus, dry common bean, hermetic storage, moisture content.

INTRODUCTION

Dry common bean (*Phaseolus vulgaris* L., Fabaceae) is the legume of greatest consumption in Mexico and part of Latin America, being a fundamental component of the daily diet of rural population. This grain has two important postharvest insect pests, the Mexican bean weevil *Zabrotes subfasciatus* (Boheman) and the common bean weevil *Acanthoscelides obtectus* (Say) both (Coleoptera: Bruchidae) (Gatehouse *et al*., 1998; López-Pérez *et al*., 2007), causing considerable qualitative and quantitative losses. Rural farmers produce dry beans and maize for their own consumption, and the remainder of their harvest is channeled insect-infested into commercial market channels. These subsistence farmers are bereft of technical assistance and economic resources, and therefore are unable to benefit from those postharvest technological advances already being implemented in more developed countries.

Water or moisture content is an important component of grain and is the crucial factor affecting the storage of the grain. The growth and proliferation of insect pests and fungi, which are the major spoilage organisms, are dependent on the available water in the grain. Biological and biochemical activities occur only when moisture is present. Hence, for safe storage of grain, both the moisture content of the grain and that of the surrounding air environment must be reduced and monitored (Jayas and White, 2003), because grains, like other stored products, are hygroscopic materials (*i.e.* they absorb and release water). These grains consist of a constant amount of dry matter, though water content will vary (Devereau *et al*., 2002). Moisture content plays a significant role in the storage of grain: when grain moisture is high, the grain heats up and mold spoilage can appear (Brewbaker, 2003). On the other hand, Calderon and Navarro (1980) and Oxley and Wickenden (1963), have found that low moisture content in grains causes a dehydrating effect on the immature stages of insect development, since insects require the moisture within the grain in order to mature.

Most grain storage losses are caused by storage with high moisture which favors the growth of insects and fungi; therefore, it is crucial that this important grain should be properly dried before its storage. Grain may also be treated with pesticides to eliminate or reduce insect infestation. However, these approaches are not suitable for use in rural areas for economic and technical reasons.

In order to avoid losses in dry bean during postharvest in rural areas, and to limit insect infestation of urban commercial grain, it is essential to have a good storage system to preserve the initial physical and nutritional quality of this important food product. Hermetic storage has been in use as long as there has been agriculture, as a means for safeguarding food for rural communities (Sigout, 1980; De Lima, 1990). Despite the time-proven benefits of hermetic grain storage including efficiency, relative low cost, and protection of the environment, however, this system has not been widely adopted in rural areas, either for economic reasons or ignorance. Even on a commercial scale in developed countries, because their storage systems have evolved to well mechanized storage structures, postharvest losses have not been brought close to hermetic storage efficiency. Nevertheless, a great amount of research has been conducted on hermetic grain storage that proves it to be an excellent alternative method for grain preservation in quantities which vary from just a few kilograms to huge volumes (Sartori and Vitti, 1991; Varnava *et al*., 1995; Moreno *et al*., 2000), despite the fact that it has not been adopted for farmers in rural areas.

The beneficial components of a hermetic storage system are based on the depletion of oxygen with the consequent carbon dioxide generation within the storage container. The important role of oxygen depletion versus carbon dioxide generation in relation to insect mortality was demonstrated by Bailey (1965). However, some researchers claim that the combination of low oxygen levels and high carbon dioxide content has an important synergetic role in hermetic grain storage for effective eradication of insect populations in grain storage containers (Calderon and Navarro, 1980; García-Perea *et al.,* 2014).

During hermetic storage of insect infested grains, oxygen depletion and carbon dioxide increase are due to the respiration of insects, fungi, and grains. This occurs according to the degree of grain moisture content and length of the storage period (Varnava *et al*., 1995; Moreno *et al.,* 2000).

In tropical areas of several countries in Central America, as well as in Mexico, hermetic storage has been used empirically, with variable results in terms of grain preservation. The handling of grain involved in the hermetic storage system appears to be poorly known or understood, and therefore it has not been properly used. In order to enhance the benefit of this system of grain storage for the rural areas, more information is needed in order to provide farmers with a simple technology that can be well-managed in their farms.

Therefore, this research was carried out to determine the moisture content of dry bean and the time of hermetic storage required to disinfect the grain and completely avoid the physical damage caused by *Z. subfasciatus*, without the use of pesticides. The purpose of this research is to pave the way to a simple technology for proper preservation of the postharvest quality of dry bean as an alternative to the application of pesticides on postharvest insect control, which is costly and risky for human health and the environment.

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 of La Unidad de Investigación en Granos y Semillas (UNIGRAS) of La Universidad Nacional Autónoma de México (UNAM), the dry common bean presented with a moisture content of 11.0% and grain germination 94% and an invasion of 4% by the fungus *Alternaria* spp. The lot of bean grain 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 *Z. subfasciatus* 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 glass jars of one gallon with 1.5 kg of dry bean and 500 weevils without sexing were introduced for oviposition. After 5 d, the insects were removed from the jars. Insects that emerged during the first five days were eliminated. The next day, those insects with less than 24h of emergence were used in the experiment.

Oxygen and carbon dioxide determination

The percentages of oxygen and carbon dioxide were determined by using digital gas analyzer equipment Illinois™, 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 content at 20.9% and carbon dioxide at 0.03%. The method for measuring levels of gases is as follows: in 15 sec, the gas analyzer takes a sample of 38 ml of air; the empty air-tight flasks have a 275 ml capacity; and the 150 g of dry beans occupy 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.

Moisture content

The moisture content of the dry bean was determined by an air-forced oven drying method of 103°C for 72 h, with two replicates of 5-10 g each per experimental unit. The moisture content percentage was calculated on the basis of the wet weight of the grain (ISTA, 1996). The moisture content in each sampling was immediately determined subsequent to the oxygen and carbon dioxide measurements.

Infestation and storage

Insect infestation and storage were achieved as follows: from a lot of dry common bean, three sub lots of 8.1 kg were each adjusted to moisture content of 10, 12, and 15%, by exposing the dry bean under three different relative humidities, maintained by means of saturated salt solutions. For 10% moisture content, the salt used was potassium carbonate (K_2CO_3); for 12%, sodium bromide (NaBr); and for 15%, sodium chloride (NaCl) (Winston and Bates, 1960). Once the desired moisture content was achieved for each sub lot, each was divided into 54 experimental units of 150 g each. The experimental units were infested with 20 adult sexing 1:1 insects and with less than 24 h post-emergence.

Twenty-seven 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 other twenty-seven experimental units 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 162 for the two storage systems, three with moisture content, and three replicates per each of the nine samplings.

All experimental units were placed in a rearing room at 28±2ºC, 75±5% relative humidity with a photoperiod of 12 h light; nine samplings were carried out every 3 d. In each one of the samplings, oxygen and carbon dioxide concentrations were recorded along with moisture content as well as the percentage of dead and alive insects of initial infestation, which were removed from each experimental unit. Those experimental units which were already insect free, were covered with wire mesh and then placed back in the rearing room for 50 d to allow for the offspring emergence from (F_1) of the eggs laid by the initial insect population and to quantify the percentage of damaged dry bean. Damage was quantified by observing the presence of exit insect holes and/or circular windows prepared by the last larval instar (Bhadriraju and Hagstrum, 1996), and taking into account the total number of dry beans remaining in the experimental unit. In addition, to confirm that not all insects complete their development and the damage remains non-visible only as internal damage which was evaluated by the use of X-rays, exposing 30 dry beans radiographed without apparent damage. Only on the experimental units of the hermetic system the larval development was evaluated within the dry bean, related to the internal damage within the dry bean.

Experimental design and statistical analysis

The experiment was carried out under a completely randomized design. 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 (α=0.05) with the Statistical Analysis System (SAS, 2002). The tables exhibit the mean values of three replicates from the original data, with the mean comparison of ranked data.

RESULTS

The statistical analysis of whole variance showed highly significant differences ($P<0.01$) between storage systems, between moisture content and also resulted in significant effect of moisture content interaction on storage systems, indicating that levels of moisture content did not follow the same trend at each level of storage systems. Variables included: oxygen, carbon dioxide, mortality, emergence and damaged dry bean percentage, during the 27 d of storage. In the case of internally damaged dry bean percentage of the experimental units of the hermetic system, there was no significant difference ($P>0.05$).

The moisture contents adjusted to dry bean, did not change significantly during 27 d evaluation in hermetic storage system for 10.0% was 10.0%; for 12.0% was 11.8%; and for 15.0% was 14.8%. In the case of open system to the desired moisture content 10, 12 and 15%, the averages were 10.5, 12.2 and 15.8%, respectively.

In the open storage system, as expected, levels of oxygen and carbon dioxide were kept constant during the storage period with 20.9 and 0.03%, respectively, at different times of evaluation.

During the evaluation time, the experimental units of the hermetic storage system showed hermetic properties, preventing gas exchange with observed decrease in oxygen levels and increase in carbon dioxide levels, for the three moisture contents evaluated (Fig. 1). In hermetic storage system at 3 d of evaluation, oxygen levels dropped from 20.9 to 14.7% on average in the three moisture contents, this value represents a reduction of 29.7%. At 9 d, for moisture contents of 10, 12 and 15%, oxygen levels were 9.8, 7.5 and 6.7%, coinciding with 100% mortality of adult insects which infested the dry bean; at the end of the storage period, oxygen levels dropped to 6.0, 3.6 and 1.8%, respectively. With regard to carbon dioxide in the atmosphere of the hermetic storage contained: at 3 d, levels increased from 0.03% to 3.3% on average for the three moisture contents, and thereafter levels of carbon dioxide increased; at the end of the storage time (27 d), experimental units with moisture contents of 10, 12 and 15% reached levels of 7.8, 10.3 and 11.8%, respectively.

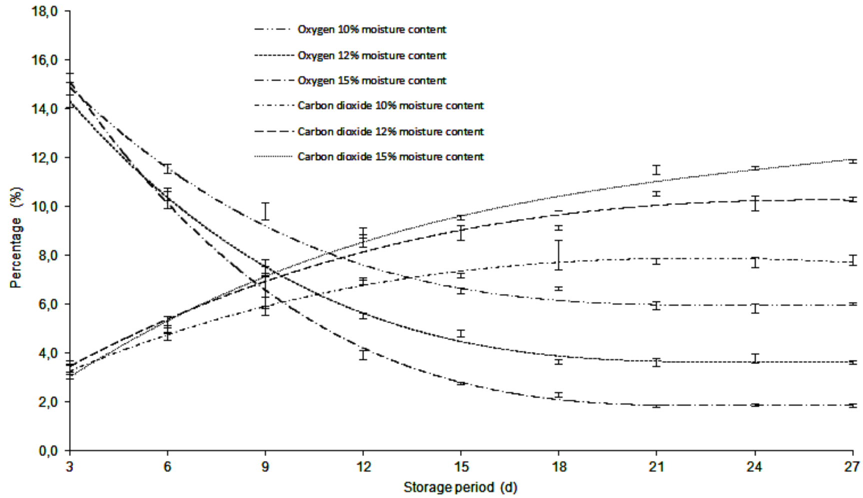

Fig. 1. Effect of the dry bean moisture content on depletion of oxygen and increase of carbon dioxide under hermetic storage by *Zabrotes subfasciatus*; fitted with a polynomial function.

In the dry beans, hermetic storage at 3 d presented an insect mortality of 27, 12 and 12%; 6 d mortality was 87, 77 and 55% for dry bean stored at 10, 12 and 15% moisture content, respectively. Insect mortality in the hermetic storage system reached 100% at day 9 for the three dry bean moisture contents (Table 1). In open storage system at 3 d for stored dry bean with 10, 12 and 15% moisture content, mortality was 13, 10 and 5%, respectively. At 6 d, mortality was 77, 58 and 38% for dry bean 10, 12 and 15% moisture content, respectively. From 9 d, mortality increased above 90% of the three moisture contents. All insects were dead from the 12 d at moisture contents of 10 and 12%, and from 15 d for the moisture content of 15% (Table 1).

The mean number of insect emergence from dry bean containing 10, 12 and 15% of moisture was 81, 93 and 96 insects respectively, after 3 d of hermetic storage, reaching maximum emergence of 129, 230 and 201 insects in the storage period of 12 d. The emergence declined in the subsequent sampling dates. No emergence occurred from dry beans containing 10 and 12% of humidity at 21 d and 27 d of storage respectively, while the emergence from dry beans with 15% of moisture content was 7 insects at the end of the stored period (Table 2).

With regard to insect emergence in the open system (non-hermetic system), the mean values were significantly higher ($P<0.01$) in the open system than insect emergence in the hermetic system, regardless of the dry bean moisture contained during stored periods, except for emergence at 3 d of storage in which no significant difference ($P>0.05$) was observed between moisture contained and stored system. At the end of the store period, insect emergence was recorded in the three dry bean moisture contents of 10, 12 and 15% which were 290, 373 and 418 insects respectively. However, in some sample dates, the insect emergence was higher than the mean numbers given previously, especially in grain stored at 21 days with 15% of moisture content, reaching an emergence of 475 insects (Table 2).

Table 1. Mortality of initial infestation of *Zabrotes subfasciatus* in stored dry bean with three moisture contents for 27 days.

System	MC (%)	Storage period (days)								
		3	6	9	12	15	18	21	24	27
Hermetic	10	27±4.4 c^{1}	87±1.7 b^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}
	12	12±1.7 c^{12}	77±3.3 b^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}
	15	12±1.7 c^{12}	55±2.9 b^{2}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}
Non-Hermetic	10	13±3.3 c^{12}	77±4.4 c^{1}	97±1.7 b^{12}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}
	12	10±0.0 c^{2}	58±4.4 c^{2}	97±1.7 b^{12}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}
	15	5±2.9 d^{2}	38±1.7 cd^{2}	92±1.7 bc^{2}	97±1.7 b^{2}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 a^{1}	100±0.0 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); d: days; MC: moisture content.

Table 2. Emergence of *Zabrotes subfasciatus* from offspring (F1), in stored dry bean with three moisture contents for 27 days.

System	MC (%)	Storage period (days)								
		3	6	9	12	15	18	21	24	27
Hermetic	10	81±5.9 c^{1}	110±4.4 b^{4}	101±7.2 b^{3}	129±6.8 a^{4}	52±2.6 cd^{4}	37±6.4 d^{4}	0±0.0 e^{6}	0±0.0 e^{6}	0±0.0 e^{5}
	12	98±7.3 cd^{1}	118±6.4 bc^{4}	112±5.2 c^{3}	230±9.2 a^{3}	155±7.8 ab^{34}	53±4.8 de^{4}	41±3.8 ef^{5}	14±1.3 fg^{5}	0±0.0 g^{5}
	15	96±8.5 d^{1}	125±4.1 c^{34}	133±6.4 bc^{23}	201±3.8 a^{34}	185±4.1 ab^{3}	97±4.9 de^{3}	63±3.5 ef^{4}	22±0.6 fg^{4}	7±1.2 g^{4}
Non-Hermetic	10	72±7.2 d^{1}	154±6.9 d^{23}	263±4.0 cd^{12}	308±11.7 b^{2}	299±6.7 bc^{2}	357±7.2 a^{12}	360±9.3 a^{3}	322±5.8 ab^{3}	290±6.6 bc^{3}
	12	102±7.0 e^{1}	182±8.4 de^{12}	253±12.1 cd^{12}	325±12.4 b^{2}	306±10.1 b^{2}	295±7.2 ab^{23}	403±10.7 a^{2}	376±5.7 a^{2}	373±9.8 a^{2}
	15	101±6.8 f^{1}	205±7.8 ef^{1}	267±5.9 de^{1}	420±10.7 b^{1}	450±8.1 a^{1}	383±4.7 cd^{1}	475±6.8 a^{1}	448±7.9 a^{1}	418±9.2 bc^{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); d: days; MC: moisture content.

The percentage of dry bean that was damaged when stored under hermetic and open systems had similar behavior with regard to the number of emergent insects (F_1) in the three moisture contents evaluated (Table 2, Fig. 2). The maximum percentage of dry bean damaged was observed, at 12 d when grain was stored in hermetic system with 16.3, 28.4 and 29.1% in the moisture content of dry bean of 10, 12 and 15% respectively. The percentage of dry bean that was damaged decreased to 0% from 21, 24 and 27 d of storage in the experimental units with moisture content of 10% and 27 d at moisture content of 12 and 15%. As expected, the percentage of damaged dry bean were above 50% in the open storage system, increasing from 15.8% at 3 d with 10% moisture content to 53.7% after 15 d of open storage with dry bean at content moisture 15%. The percentage of dry bean damaged when stored under hermetic and open systems is highly correlated with the number of insects emergent in the three moisture contents evaluated in this study. The correlation coefficients (*r*) in hermetic system were 0.96, 0.97 and 0.95 for 10, 12 and 15% moisture content, respectively; in non-hermetic systems *r* values were 0.97, 0.91 and 0.98 for 10, 12 and 15% moisture content, respectively (data not shown).

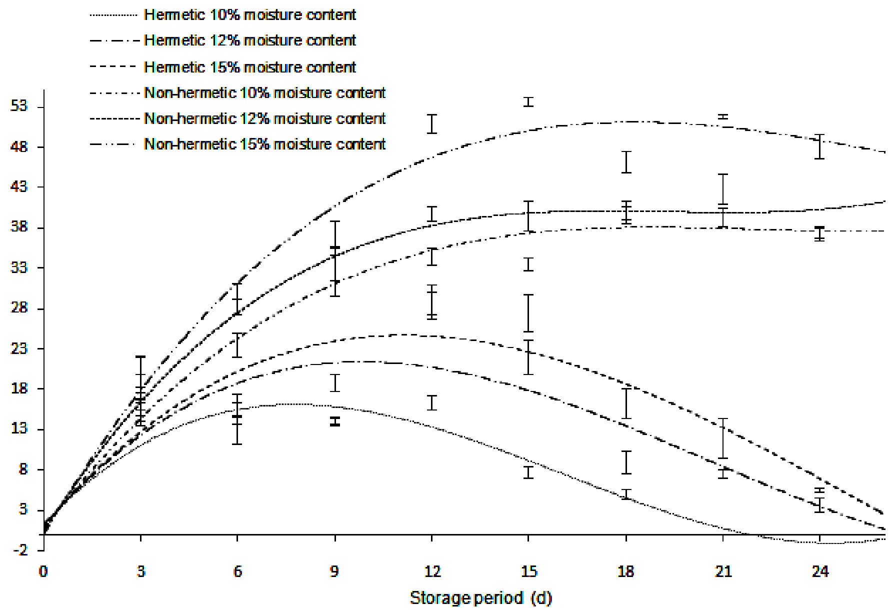

Fig. 2. Effect of moisture content under both storage systems on dry bean damage by *Zabrotes subfasciatus*; fitted with a polynomial function.

Internal damage was observed in dry beans hermetically stored in the three moisture contents, showing no significant difference (*P*>0.05). Percentages of internal damage of 1.6% to 3 d storage were observed up to 7.1% at 12 d with 15% moisture content (Table 3). Internal damage evaluation was carried out on samples of dry beans exhibiting no apparent damage; it was done in order to observe larval development within the grain in which the life cycle was incomplete cycle, and to handle this as completely sound grain.

DISCUSSION

The desired dry bean moisture content in hermetic storage was quite similar to the adjusted content. This was, in part, due to the short storage period, the relatively low dry bean moisture content and the rapid oxygen depletion, all of which were not factors conducive to promoting insect and fungi activities, resulting in an increase on the initial moisture content. However, the moisture content of dry bean that was non-hermetically stored was higher than those of grain which was hermetically stored. This can be attributed to insect activity that was higher than that in hermetic storage. Similar results with the larger grain borer, *Prostephanus truncatus* (Horn) were reported by Quezada-Viay *et al.* (2006) and García-Perea *et al.* (2014) with *A. obtectus*. It is well known that insect respiration generates water and carbon dioxide, as well as oxygen (Christensen and Kaufmann, 1969; Moreno *et al.*, 2000). Regarding the low presence of storage fungi, the amount of invasion and type of fungi are typical factors for grain that has been stored for a short period and under low moisture conditions (Christensen and Kaufmann, 1969).

Table 3. Internal damage of dry bean with three moisture contents, under hermetic storage for 27 days.

System	MC (%)	Storage period (days)								
		3	6	9	12	15	18	21	24	27
Hermetic	10	81±5.9 c^{1}	110±4.4 b^{4}	101±7.2 b^{3}	129±6.8 a^{4}	52±2.6 cd^{4}	37±6.4 d^{4}	0±0.0 e^{6}	0±0.0 e^{6}	0±0.0 e^{5}
	12	98±7.3 cd^{1}	118±6.4 bc^{4}	112±5.2 c^{3}	230±9.2 a^{3}	155±7.8 ab^{34}	53±4.8 de^{4}	41±3.8 ef^{5}	14±1.3 fg^{5}	0±0.0 g^{5}
	15	96±8.5 d^{1}	125±4.1 c^{34}	133±6.4 bc^{23}	201±3.8 a^{34}	185±4.1 ab^{3}	97±4.9 de^{3}	63±3.5 ef^{4}	22±0.6 fg^{4}	7±1.2 g^{4}
Non-Hermetic	10	72±7.2 d^{1}	154±6.9 d^{23}	263±4.0 cd^{12}	308±11.7 b^{2}	299±6.7 bc^{2}	357±7.2 a^{12}	360±9.3 a^{3}	322±5.8 ab^{3}	290±6.6 bc^{3}
	12	102±7.0 e^{1}	182±8.4 de^{12}	253±12.1 cd^{12}	325±12.4 b^{2}	306±10.1 b^{2}	295±7.2 ab^{23}	403±10.7 a^{2}	376±5.7 a^{2}	373±9.8 a^{2}
	15	101±6.8 f^{1}	205±7.8 ef^{1}	267±5.9 de^{1}	420±10.7 b^{1}	450±8.1 a^{1}	383±4.7 cd^{1}	475±6.8 a^{1}	448±7.9 a^{1}	418±9.2 bc^{1}

Original means of three replicates ± standard error; MC: moisture content.

Moreno *et al.* (2000), working with the maize weevil, *Sitophilus zeamais* (Motschulsky) on hermetically stored maize under laboratory conditions, found that at 9 d of storage, the oxygen content was 1% with 100% insect mortality. García-Perea *et al.* (2014), working with *A. obtectus* also under hermetically stored conditions for dry bean found that at 9 d of storage, oxygen and carbon dioxide levels did not allow the development of insect between 0.5-3.5% and 8.9-9.7%, respectively. These results are also quite in agreement with Oxley and Wickenden (1963), who mentioned that oxygen levels around 2% in hermetic storage causes mortality to the granary weevil, *S. granarius* (Linnaeus).

In hermetic storage, oxygen depletion and carbon dioxide generation were fluctuating throughout the storage period, but it was not until the end of the storage period that significant differences could be clearly observed amongst the storage atmospheres for dry beans with different moisture content. However, in this study, insect adult mortality of initial infestation of *Z. subfasciatus* occurred with relatively higher oxygen contents (Fig. 1 and Table 1), than those mentioned by the researchers cited in the previous paragraph. At 9 d of hermetic storage, insects were all dead with relatively high oxygen levels of 9.8, 7.5 and 6.7% for dry bean stored with moisture contents of 10, 12 and 15% respectively; and average oxygen depletion was around 62% (Fig. 1).

These results revealed that all insects do not have the same degree of sensitivity under low oxygen hermetic storage conditions. Also, it should be taken into account, that *Z. subfasciatus* has a short longevity of 12 d and are more active than *A. obtectus* and that these factors may be involved in the sensitivity of this insect to the storage atmosphere oxygen level, in addition to a possible desiccation effect, as noted by Navarro (1978). With regard to this, Emekci *et al.* (2002), found that the red flour beetle, *Tribolium castaneum* (Herbst) had higher respiration activity in an atmosphere containing 3 and 5% oxygen than in a normal atmosphere; and it has also been stated that there is a high insect mortality attributed to desiccation caused by low oxygen content and low grain moisture content with consequently low intergranular humidity (Navarro, 1978).

In this study, the oviposition of *Z. subfasciatus* was observed at 3 d of storage in contact with the dry bean in the three different moisture contents storage conditions. Howe and Currie (1964) reported that adults of this species are sexually mature, and ready for copulation immediately following emergence. The same authors, suggested that *Z. subfasciatus* females lay most of their eggs at the beginning of the oviposition period in order to avoid risk factors, such as predation or competition, since this insect has a short life cycle.

It has been stated that most storage losses of grains are due to an excess of moisture content; however, unpublished results demonstrate that the quite low moisture contents so often found in open storage of grains in underdeveloped regions are at a greater risk (around 8%) for the auto-consumption harvest, since it is well known that insects are able to develop under very low grain moisture content

The role of grain moisture content in the preservation of hermetically stored dry bean infested with *Z. subfasciatus* was mainly observed in the emergence of the offspring (F_1) in which moisture content of 10% from 21 to 27 d revealed no emergence combined with low levels of oxygen between 5.8 to 6.0% and carbon dioxide between 7.70 to 7.80%. For moisture content of 12%, oxygen and carbon dioxide levels were 3.6 and 10.27%, respectively. For storage of dry beans with 15% of moisture, at 27 d there was an emergence of 7 insects with a percentage of visible damage 1.6%, which is the lowest percentage found to at the end of the experiment. This means that *Z. subfasciatus* was more active at 12 and 15% than at 10% moisture content, consequently causing more grain damage.

Furthermore, it has been shown that, under hermetic storage, a low dry bean moisture content of 10%, in combination with an oxygen concentration, around 6%, and a short storage period of 27 d, killed the eggs, larvae and adults of *Z. subfasciatus*. The dry bean remained free of holes, meaning that the adult insects did not emerge from the dry beans, and also the eggs and larvae were unsuccessful in completing their stage of the life cycle.

With this research, it is demonstrated that the moisture content of dry bean plays an important role in the preservation of grain that is hermetically stored, since with oxygen levels of up to 6.0%, with moisture contents of 10 and 12% there was no insect emergence. However, for 15% moisture content found in all time emergence sampling, even with low oxygen levels of 1.8%. This agrees with Calderon and Navarro (1980) and Oxley and Wickenden (1963), who mentioned 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.

The percentage of internal damage to the dry bean due to the development of the larvae of *Z. subfasciatus* shows that the visible physical damage does not match the percentage of dry bean internal damage, since the latter is not seen by the naked eye, but rather through the use of X-rays where it can be observed that there is damage by underdeveloped larvae, which consume part of the cotyledons and cause pollution with their bodies and other substances, and which are consumed in the preparation of this food.

As expected, under non-hermetic conditions, the number of emerging insects greatly increased throughout the storage period, causing great physical damage, up to 53.7%; this is the situation that often occurs in rural areas of developing countries.

The basic advantage of hermetic storage for developing countries lies in its simplicity and effectiveness, reducing the need for dangerous insecticidal applications or expensive and risky fumigations which require costly resources and technical knowledge generally not accessible in rural areas and which contribute to serious is public health and environmental problems.

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