Effect of *Sitophilus zeamais* and *Aspergillus chevalieri* on the oxygen level in maize stored hermetically

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Abstract

Maize grain of hybrid AN 447 was: (a) infested with *Sitophilus zeamais* and infected with *Aspergillus chevalieri*; (b) infested with *S. zeamais*; (c) infected with *A. chevalieri*; and (d) grain free of insects and fungus (control); the treatments were stored for 30 days at 26°C and 15% moisture content, under hermetic and non-hermetic conditions to monitor the oxygen concentration, insect mortality, insect offspring, grain germination, and fungal growth. The oxygen was depleted to 0% after 6–9 days in those treatments infested with insects, whereas the same oxygen level was reached after 24 days in grain with the storage fungus alone. The oxygen level gradually decreased to 8.4% after 30 days in the control treatment. All insects were dead after 6 days in grain with insects and fungus, and after 12 days in grain with insects alone. A low mortality rate (1.5–3.5%) occurred in equivalent treatments of the non-hermetic conditions. Because oxygen was depleted to 0% after 6 to 9 days in those treatments infested with insects, the weevils of both infested treatments under hermetic conditions produced a significantly lower number of offspring compared with those in the non-hermetic conditions. Under hermetic conditions in grain treated or not treated with fungicide, the storage fungus *A. chevalieri* invaded a low percentage of grains. A low percentage of fungal invasion occurred in grain stored under non-hermetic conditions also, where the decreased moisture content did not favor fungal growth. The grain germination of those treatments stored under hermetic conditions was significantly lower than those treatments stored under non-hermetic conditions. The insects were the main oxygen consumers, followed by the fungus and finally by the grain. Under sealed storage conditions, insects and fungus combined forces to deplete the oxygen of hermetically stored maize, creating an unfavorable atmosphere for their own survival. © 2000 Elsevier Science Ltd. All rights reserved.
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1. Introduction

The human population explosion of developing countries worldwide is creating an unprecedented demand for greater production of food grains. Maize is the most important grain for the Mexican population, as well as for many other developing countries. In these countries, maize is produced primarily on small farms. The farmers retain part of the harvest for their own consumption, eventually selling the remainder to the urban population. The preservation of grain quality at the farm level, therefore, is of great importance. This is a difficult task, however, particularly in those tropical areas where drying and storage technologies are deficient or completely lacking.

Stored-grain insects and fungi cause severe quantitative and qualitative losses, which have grave implications for the availability of food, especially in those areas of the world where grain storage is poorly managed due to the lack of proper knowledge and technology (Sauer et al., 1992; Harein and Davis, 1992). In order to prevent excessive losses in quality and quantity during grain storage, the practices of artificial drying and cooling aeration, or the use of insecticides, are techniques most commonly utilized by countries which can afford them (Hall, 1980; Cuperus et al., 1986; Arthur, 1994; Bell, 1993; Brook, 1992; Harein and Davis, 1992). Developing countries, however, have resorted primarily to the use of insecticides.

In these countries, the indiscriminate and improper use of insecticides to control the proliferation of stored-grain insects, has resulted in the development of resistant strains of insects which require an increase in dosage as well as frequency of applications to keep them under control (Parkin, 1965; Dyte, 1970; Champ and Dyte, 1976). Furthermore, the use of some insecticides may be an ecological (USEPA, 1993) and a public health risk. When farmers in developing countries have problems with stored-grain insects, in the absence of state-of-the-art technical knowledge, they tend to buy any insecticide which is readily available, including such toxic chemicals as lindane, parathion, methyl parathion and carbaryl—insecticides which were frequently used in Mexican rural areas only a few years ago to control stored-grain insects. It is therefore imperative to develop alternative methods that are economically feasible and ecologically oriented to control stored-grain insects and fungi—hermetic storage may be one of those alternative methods.

Millennia of agricultural practice, in a world long reliant upon the storage of grains, has proven hermetic storage to be a strong alternative method for grain preservation ranging from a few kilograms to vastly larger amounts (Hyde, 1965; Sigout, 1980; Sartori and Vitti, 1991; Kawasugi et al., 1994; Varnava et al., 1995). In the tropical areas of some countries of Central America as well as in Mexico, hermetic storage of maize for human consumption has been empirically used. To improve hermetic storage performance, more information is needed pertaining to maize stored under those moisture conditions encountered during harvesting and storage by farmers in tropical areas. Since the success of a hermetic storage system is based on oxygen depletion and carbon dioxide evolution in the storage container, it is important to determine the impact of factors which influence these parameters. Therefore, research was
conducted to determine the roles of insect, fungal and grain respiration on oxygen levels, and the effect of modified atmosphere on insects, fungi and grain.

2. Materials and methods

Maize grain of the commercial hybrid AN-447 with 89% germination, a moisture content (m.c.) of 12% and a 2% rate of infection by the storage fungus, *A. chevalieri* Mangin, was utilized in storage tests. Moisture content was determined by drying replicate portions of 5–10 g each of whole grain at 103°C for 72 h with percentages calculated on a wet-weight basis (USDA, 1976).

To determine the percentage of grain germination, 100 seeds of maize of each of three replicates were germinated at 25°C between moist paper towels. The germinated grains were counted after 7 days (a grain was considered germinated when the tip had protruded from the grain). Oxygen from the hermetic storage flasks (250 ml with 150 g of grain) was measured by using an oxygen analyzer, Servomex Model 570 A (Crowborough, Sussex, UK). At each sampling period an air sample of a set volume was obtained from each replicate unit through a rubber disk placed on the covers, by means of a syringe connected to the oxygen analyzer. The hermetic flasks were sampled only once to test the oxygen levels—the creation of a vacuum inside the flask did not therefore present a problem. The kind and amount of fungal infection were determined by plating 50 grains of each replicate on 2% agar, 2% malt and 6% salt (MSA) medium. The grains were surface-disinfected with a 2% NaOCl solution for 1 min, rinsed in sterile water prior to planting, and incubated for 7–10 days at 25°C, until the colonies could be properly identified and counted (Raper and Fennell, 1965).

Adults of the maize weevil, *S. zeamais* Motschulsky, were collected in 1995 from maize silos of a rural area and were maintained in the laboratory, under rearing room conditions of 25°C, 70% r.h. and an 18–6 h light and dark (L–D) photoperiod, as a stock culture in a non-sterilized, starch-rich maize variety which was free of pesticides. To obtain an insect population of known age, 100 adult weevils (both sexes) were transferred from the stock culture to a 1 liter jar containing maize grain with 12.5% m.c. The adults were allowed to oviposit for a 24 h period on the grain, and subsequently removed. The jars were kept at 25°C, 70% r.h., in an 18–6 h L–D photoperiod. The adults which emerged from these cultures were used in this experiment to study the effect on the oxygen concentration of the hermetic storage atmosphere.

The storage test was conducted as a completely randomized factorial experiment with three factors: (a) storage system (hermetic and non-hermetic storage); (b) treatments (four); and (c) storage period (10 sampling periods) with three replicates of 150 g of grain each per treatment for each sampling time. The moisture of the grain to be stored was adjusted to 15% by the addition of water (Pixton, 1982). The four treatments were: (a) grain + insect + fungus (effect of grain, insects and fungus respiration on oxygen consumption); (b) grain + fungus (effect of grain and fungus on oxygen consumption); (c) grain + insects (effects of grain and insects on oxygen consumption); and (4) control, free of insects or fungi (effect of grain respiration alone on oxygen consumption).

In order to avoid fungal growth on those treatments requiring the absence of fungus, the maize grain was treated with the fungicide chlorathalonil. To determine the toxicity of chlorathalonil to the maize weevil *S. zeamais*, an experimental test was conducted. Thirty glass
jars containing 150 g of maize grain were treated with 750 ppm of the active ingredient of the fungicide. Another 30 similar jars containing non-treated grain were used as controls. Each one of the 60 jars was infested with twenty 10-d-old unsexed adults of the maize weevil. The jars were kept for 30 days under rearing room conditions of 25°C, 70% r.h., and 18–6 h L–D photoperiod. Mortality of the maize weevil was recorded at 10, 20 and 30 days by checking 10 jars (replicates) of treated and non-treated grain. Mortality data were analyzed with an ANOVA test by using the statistical program Statview in MacIntosh. The statistical test did not reveal significant differences in mortality rates of the insects infesting treated and non-treated grain for the three exposure periods. It was therefore concluded that the fungicide was not toxic to the maize weevil.

To establish the first treatment, the grain of each experimental unit was infested with 20 unsexed adult insects of *S. zeamais*. The grain of this treatment was not artificially inoculated with storage fungi as maize always carries spores of these fungi. The second treatment consisted only of grain with the natural fungal inoculum. The third treatment was accomplished by infesting the grain of each experimental unit with 20 adult insects. The maize grain of these experimental units was treated with 750 ppm (active ingredient) of the fungicide chlorathalonil to prevent fungal development. The fourth treatment consisted of maize grain treated with the fungicide chlorathalonil to prevent the development of storage fungi. Two hundred and forty experimental units of 150 g of maize grain each were used in this storage test. One hundred and twenty experimental units were stored under hermetic conditions in sealed glass flasks (the plastic covers had a rubber septum to permit air sampling). The initial oxygen concentration of the sealed flasks was 20.9%.

Another 120 experimental units were stored under non-hermetic conditions in glass flasks covered with metal wire screens to keep the insects inside. The experimental units were randomly placed in the storage chamber at 26°C and 70% r.h. The first sampling in both storage systems commenced on the third day and thereafter every 3 days during the course of 30 days of storage. For each sampling period, the oxygen content of the hermetic containers was measured, as well as the moisture content of the grain, number of dead insects, the emergence of insects, microflora and germination rates of the grain.

After registering the number of dead insects and removing the live ones, the grain of each experimental unit, including both hermetic and non-hermetic units, was divided into two halves. One half was used to determine moisture content, germination and microflora. Since it was suspected that live insects may have laid eggs, the other half was incubated at 70% r.h., and 26°C for 30 days to observe the possible emergence of insects, which was recorded for each particular sampling period. Prior to the statistical analysis, an arcsin transformation of the oxygen data was performed and also a square root transformation of the dead insects and insect emergence data. Duncan’s multiple range test was used to determine statistical differences among treatments (Steele and Torrie, 1980).

### 3. Results

The analysis of the oxygen variance, insect mortality, insect emergence, grain germination and fungus data all showed significant differences ($P \leq 0.01$) within storage systems, storage
periods and treatments. There were also significant differences in interactions between storage systems/storage periods, storage systems/treatments, storage periods/treatments and storage systems/storage periods/treatments for insect mortality, insect emergence, and fungus. However, neither differences in oxygen nor germination data were detected in the interaction of storage periods/treatments nor in germination data in the interaction storage system/storage periods/treatments ($P > 0.05$). In all the hermetic storage treatments, oxygen levels decreased from ambient levels in all treatments (Fig. 1). After 3 days of storage, the oxygen level of the treatments ranged from 1.5% ($\pm 0.17$) to 16.6% ($\pm 0.03$). The treatments which exhibited the most rapid and sizable decrease of oxygen were grain + insects + fungus, and grain + insects (Fig. 1).

Those treatments in which insects and fungus were present, either together or alone, demonstrated a rapid decrease in oxygen level. By the third day of storage, grain + insects + fungus, had an oxygen level of 1.5% ($\pm 0.17$) and the grain + insects had an oxygen level of 8.1% ($\pm 0.0$) (Fig. 1). After only 6–9 days of storage, the oxygen levels were 0% in both treatments. Grain infected with fungus that was insect-free (grain + fungus) had an oxygen level of 15.6% ($\pm 0.61$) at 3 days of storage and 12.6% ($\pm 0.55$) at 15 days, and thereafter the oxygen level rapidly decreased to 0.0% at 24 days. The oxygen level in grain without insects and fungus (control), gradually decreased until 24 days (13.7% $\pm 0.67$), reaching an 8.4% ($\pm 1.2$) oxygen level at the end of the 30 days.

Under hermetic conditions, after 6 days of storage, insect mortality in the treatment grain + insects + fungus was 100% (Fig. 2). Insect mortality in the treatment grain + insects was 10% ($\pm 4.4$) at 6 days and 100% at 9 days. However, in grain stored under non-hermetic conditions, insect mortality was very low. In grain + insects + fungus, at 27 and 30 days of storage, there was a 5% insect mortality. In the treatment grain + insects, insect mortality was 5% at 21, 24, 27 days of storage and 15% ($\pm 6.0$) at 30 days of storage.

![Fig. 1. Oxygen levels in maize stored under hermetic conditions.](image)
After recording insect mortality and taking samples for measuring moisture content, germination and fungus at each storage period, the rest of the grain of the experimental units was incubated 30 days at 70% r.h. and 26°C to allow the insect eggs to hatch and the larvae to develop. Under the hermetic conditions, the average number of emerging insects was low and decreased very rapidly (Fig. 3). The treatment grain+insects+fungus yielded 12 (±0.88) insects emerging from grain stored 3 days, 18 (±6.3) insects from grain stored 6 days, 3 (±2.0) from grain stored 9 days, 1 (±0.3) from grain stored 12 days and none thereafter. The
grain + insects had 16 (±6.4) insects emerging from grain stored 3 days, 21 (±1.7) insects from grain stored 6 days; 10 (±1.4) insects from grain stored 9 days and 0 thereafter.

As expected, under non-hermetic conditions, the average number of emerging insects increased throughout the storage period (Fig. 3). The grain + insects + fungus yielded 28 (±6.5) emerging insects from the third day of storage, and 229 (±16.3) from the last sampling period. The grain + insects produced 19 (±3.7) emerging insects from 3 days of storage and 172 (±32.0) at the end of the storage period.

Table 1 shows the seed germination rates of all treatments in the hermetic and non-hermetic storage after 30 days. Under hermetic conditions, the germination rates for all treatments were lower than the 89% initial germination, ranging from 55 to 73%. On the other hand, during the same storage period, the final germination rates of the grain stored under non-hermetic storage ranged from 80 to 91% (Table 1).

Grain stored under hermetic conditions, grain + insects + fungus exhibited 13% average invasion by *A. chevalieri* with the highest percentage at 18 days (27% ±3.7) declining to 1% at 30 days of storage. Grain + fungus showed a 44% average of grain invaded by members of this group, with a maximum percentage of 89% (±29.6) at 18 days, and 18% (±9.0) at the end of the storage period. On the other hand, there was poor fungal growth in grain + insects, as well as in grain alone, with averages of 1% and 3% of grains invaded by the storage fungus, respectively.

In grain stored under non-hermetic conditions, grain + insects + fungus 25% of grain was invaded by *A. chevalieri* with the highest percentage at 24 days (47% ±22.7) and at the end of 30 days (38% ±9.1). Grain + fungus had 11% of grain invaded by fungi, reaching a maximum percentage of 30% (±16.6) at 18 days and declining to 7% (±6.7) at 30 days. In this case, as well as in the case of hermetic storage, there was a poor fungal growth in grain + insects as well as in grain alone, with averages of 1% of grain invaded by the storage fungus for both treatments (Fig. 4).

The moisture content of the grain stored under hermetic conditions remained practically the same as the initial moisture content, ranging from 14.8 to 15.0%; however, under non-hermetic conditions the grain lost moisture through the storage period, with final moisture contents ranging from 10.9 to 12.1%.

Table 1
Germination of maize grain stored 30 days at 26°C under two storage systems with an initial moisture content of 15.0% and germination of 89%a

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Hermetic (Mean ± SE)</th>
<th>Non-hermetic (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain + insects + fungus</td>
<td>68 ± 5.0 a b</td>
<td>80 ± 6.0 b</td>
</tr>
<tr>
<td>Grain + fungus</td>
<td>55 ± 2.5 c</td>
<td>91 ± 2.4 a</td>
</tr>
<tr>
<td>Grain + insects</td>
<td>73 ± 0.7 a</td>
<td>81 ± 5.2 b</td>
</tr>
<tr>
<td>Grain</td>
<td>68 ± 1.2 a b</td>
<td>91 ± 1.8 a</td>
</tr>
</tbody>
</table>

a Entries in columns followed by different letters are significantly different (Duncan ≤ 0.05).
4. Discussion

The rapid oxygen depletion corresponded to those hermetic treatments in which the grain was infested with insects and fungus, either in combination or by themselves; whereas in grain free of insects and fungus (control) oxygen was not completely depleted at the end of the storage period, it had an atmosphere with an 8.8% oxygen level. In this experiment, the insects consumed more oxygen than fungus and grains by themselves. Under more moist conditions, this relationship would probably change. The results of this experiment are in concordance with those of Oxley and Wickenden (1963), who stated that stored grain insects will perish if the oxygen level of a hermetic storage atmosphere should fall to approximately 2%.

The consumption of oxygen by the storage fungus seems to be due mainly to the spore germination process, activated by the initial 15% moisture content of the maize grain; and thereafter by the growth of the mycelium, until the atmosphere, lacking oxygen reaches a fungistatic level. Seitz et al. (1982), in their studies of dry matter loss, stated that both grain and fungal respiration contribute to dry matter loss, but that fungi were the major contributors to carbon dioxide production. Sauer et al. (1992), stated that corn seeds with 15–16% m.c. had an oxygen consumption of 10% and the remainder (90%), was due to fungal respiration.

The level of oxygen in the different treatments of the hermetic system defined insect mortality as well as insect emergence, grain germination and fungal development. Total mortality of the adult maize weevils (S. zeamais, at 6 days in grain + insects + fungus, and at 9 days in grain + insects) clearly demonstrates the lethal effect of a storage atmosphere depleted of oxygen. The 100% mortality rate occurred faster in grain + insects + fungus due in larger part to the combined action of the three elements of the hermetic storage system than to the combination of grain + insects, grain + fungus or grain alone. It is already well-established that the extent of oxygen depletion largely depends on the elements of the storage system, such as
the quality and quantity of the grain, moisture content, size of the insect population(s), and fungal inoculum (Krishnamurthy et al., 1986).

In contrast to the hermetic storage, extremely low insect mortality occurred during the open storage period of the treatments grain + insects + fungi and grain + insects. The differing effects on insect mortality between the two tested systems demonstrates the lethal consequences of a lack of oxygen in combination with a high level of carbon dioxide due to the respiration of grain, insects and fungi in a hermetic storage system (Krishnamurthy et al., 1986; Spratt, 1984; Soderstrom et al., 1996). Although the carbon dioxide levels were not measured in this experiment, the effects of this gas should be kept in mind, since Calderon and Navarro (1980) demonstrated the synergistic effect of oxygen depletion with high levels of carbon dioxide for insect control. Bailey (1955, 1965) achieved total control of various stored-grain insects in a low oxygen atmosphere in combination with a high concentration of carbon dioxide.

The low rate of offspring emergence in treatments grain + insects + fungus, and grain + insects under hermetic storage was the result of the oviposition performed during the first days of storage by those adult females which survived the early days (up to 6 days) of low oxygen levels, from the initial 20.9% to 0 and 0.1%, respectively. It has been demonstrated that the immature stages of many species exhibit higher tolerances than adults to hermetic storage conditions (Annis, 1987). During the first days of storage, insects and eggs were exposed to a non-lethal atmosphere; once the atmosphere became lethal, due to the lack of oxygen and the presence of carbon dioxide, those eggs already laid or young larvae were unable to develop. High emergence occurred in both treatments within an open system.

As expected, at the end of the storage period under hermetic conditions the germination rates for all treatments were lower than the original germination rates. Previous studies have demonstrated that maize seed with a relatively high moisture content, above 15%, will not have a preservation period of more than a few weeks under a hermetic system; and also that maize with a moisture content of 14% when stored hermetically, demonstrates a germination profile which is quite similar to seed stored in an open system (Moreno et al., 1988). Hyde (1965) also mentioned that grain with a moisture content of 14% or less maintained viability at a high level for a long period. According to those authors, hermetic storage of grain for planting has a relatively short life when the moisture content is above 14%; therefore, this system cannot be considered for a long storage period, unless the seed has a low moisture content and is kept at low temperature, as is the case in seed germplasm banks. In this experiment, under hermetic conditions, the germination loss can neither be attributed to insects (since no mechanical damage by weevil feeding was observed), nor to the activities of fungus (since the percentage of internally invaded grains was too low). It would appear that germination loss was due primarily to unfavorable storage moisture and oxygen levels. In the case of non-hermetic storage, germination rate was almost as high as the original one, which underlines the deleterious effect of the hermetic conditions upon the viability of most grain embryos.

The moisture content of hermetic storage conditions remained constant throughout the storage period (14.9–15.1%); therefore the grain had enough moisture to allow germination of the storage fungal spores which consumed the oxygen in the germination process, with a following light invasion of grains (up to 6% of internally invaded grains, in the treatment grain + fungus) due to the subsequent lack of oxygen (Fig. 1). These results are supported by
Richard-Molard (1988), who stated that fungal growth and sporulation slow down as the oxygen is progressively depleted; and that below an oxygen level of 1%, even in moist grains, no activity of the fungi can be observed, even when the spores of some species can survive for prolonged periods of suspended life. Also Banks (1981) concluded that the evidence overall demonstrates that an atmosphere of low oxygen and high carbon dioxide inhibits the growth of fungi on moist grain, but does not kill them.

The poor fungal development in any of the two non-fungicide treatments (grain + insects + fungus, and grain alone) in the non-hermetic system was due to the low moisture content of the grain, which gradually decreased from the initial 15% to moisture contents between 12.1% and 10.9% (maintaining a steady relative humidity of 70% within the storage chamber was difficult to achieve). In spite of the decreased moisture content in the open storage system, the insect mortality was significantly lower compared to the hermetic system, and the final emergence of insects was much higher than in sealed storage. These results, as well as those of Banks (1981) and Moreno et al. (1988), reveal the potential advantage of storing grain free of storage fungi under hermetic conditions.

According to these results, the insects were the main consumers of oxygen, followed by the fungus, and finally by the grain (Fig. 1). In regard to this, Singh et al., (1976) found that adult insects of *Sitophilus oryzae* (L.) had an oxygen consumption of 100 μl/adult/day. Insects and fungi combine forces to deplete oxygen, creating a most unfavorable atmosphere for themselves. Further research is needed to obtain more information about the way the different stored-grain insects and the storage fungi may be used to produce a lethal atmosphere in a short period of time without risk to the nutritional and hygienic qualities of the grains, especially for grain stored in small quantities in rural areas.

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