

# Phosphine Concentration Change During Fumigation In Hermetic Plastic Bags (Silobags)

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## ABSTRACT

The goal of this experiment was to develop and test a suitable methodology for treatment of grain stored in plastic bags with phosphine. The treatment should guarantee a 200 ppm concentration of phosphine during at least 5 days. Two plastic bags storing about 200 tonnes of dry wheat each were used for the test, and a third bag was used as control. The first treatment consisted of adding 1 tablet of aluminum phosphide per cubic meter (3 g/m<sup>3</sup> of aluminum phosphide or 1 g/m<sup>3</sup> of phosphine) and the second treatment duplicated the dosage. The tablets were inserted each 5 linear meters along the bag. Additionally, cages containing live insects (*Sitophilus oryzae*) were inserted in different locations of the bag. Phosphine concentration was daily measured in different locations of the bag during 10 days. After the treatment, the cages with insects were inspected and insect mortality was recorded. The main results indicated that the treatment with the lowest phosphine concentration (1 g/m<sup>3</sup>) was enough to reach the target concentration during 5 days in most of the bag. The area close to the end of the bag resulted with lower than desired phosphine concentration, indicating lack of airtightness in that sector. The areas closer to the location where the aluminum phosphide tablets were inserted resulted with higher phosphine concentrations than other areas. However, spacing the application points each 5 m along the bag resulted in an efficient gas distribution. Insect mortality was 100% in both treatments, while in the control mortality was between 13 and 33%.

**Keywords:** Phosphine, time of exposition, monitoring, insect control, storage, grain.

## 1. INTRODUCTION

The plastic bag (or silobag) is a temporary storage system, widely used in Argentina for the storage of various grains (wheat, soybeans, corn, sunflower, etc.). The evolution of plastic bags shows a rapid increase since year 2000 (5 million tonnes) to 2005 (21,4 million tonnes), and reaching in the year 2007/2008 a maximum of 40 million tonnes (Casini, 2008).

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Most of the grains stored in bags belong to farmers. However, the industry and grain elevators held 5 years ago about 20% of the grain stored in bags (Vicini, 2006), and today that proportion that increased dramatically (Figure 1).



Figure 1. Silobags full of grain in a field close to an elevator.

### **1.1 Sources of insect pest infestation in the plastic bag**

Farmers typically store the newly harvested grain in bags that are placed on-farm. This involves minimal handling from harvest, to storage and skipping the grain out of farm, which reduces sources of insect infestation. Some studies indicated that in the Southeastern of the Buenos Aires province (Argentina), no presence of insect pests in crops of maize and wheat in the field previous to harvest was detected. At the same time, no insect were detected in silobags of soybeans (Ochandio, 2008) and wheat (PRECOP, unpublished). However, studies in Central and Northern provinces of the country (higher temperature) indicated that a proportion of the crop could be infested. Subramanyam and Hagstrum (1995) observed that the insect infestation in the fields is generally low, but increases with proximity to the grain storage facilities, which serve as a source of reinfestation of insects to field crops. Grain elevators and the industry generally store grains from many different locations in bins or flat storage structures for a more or less extended period of time. Due to these circumstances, the grain is more likely to be infested with insects during storage. On the other hand, when grain is stored in silobags, the insect infestation during storage is unlikely to occur, since the bag is an effective physical barrier to prevent infestation during storage (Cardoso et al., 2007).

### **1.2 Effect of storage conditions of silobag on the mortality of insect**

Several authors (Rodriguez et al. (2005), Casini (2009), Bartosik et al. (2008)) quote the plastic bag (Figure 1) as a system of modified atmosphere, where the oxygen ( $O_2$ ) in the interstitial air of the grain mass is partly consumed by the aerobic respiration of microorganisms, insects and grain, resulting in the increase of the carbon dioxide ( $CO_2$ ) concentration. Bartosik et al. (2008)

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proposed that the composition of O<sub>2</sub> and CO<sub>2</sub> in the bag depends on the balance between respiration (O<sub>2</sub> consumption and CO<sub>2</sub> generation), the entry of O<sub>2</sub> into the bag and the loss of interstitial CO<sub>2</sub> to the outside (due to the plastic permeability or holes in the plastic cover). The lethal effect of hypoxia (O<sub>2</sub> < 21%), anoxia (lack of O<sub>2</sub>) or hipercarbia (CO<sub>2</sub> > 0,03%) in insects has been extensively tested (Bailey, 1965; Banks and Field, 1995). However, the combined effect of an atmosphere with low O<sub>2</sub> and higher CO<sub>2</sub> (hypoxia and hipercarbia) as the one obtained on the plastic bag remains controversial. Annis and Morton (1996) argued that there is an antagonistic effect between a low concentration of O<sub>2</sub> and a high concentration of CO<sub>2</sub>, with a reduction in the mortality of insects when the CO<sub>2</sub> concentration increases. However, the most accepted hypothesis holds that there is a synergistic effect when CO<sub>2</sub> is added to environments with a low amount of O<sub>2</sub> (Calderon and Navarro, 1980; Donahaye et al., 1996). Apparently, this effect would occur at moderate levels of CO<sub>2</sub> (5-15%) but not with higher levels of this gas (Mitcham et al., 2006). Research conducted in Argentina on the control of insects in plastic bags were focused mainly on *Sitophilus oryzae*, considered a pest of great importance and one of the most tolerant to CO<sub>2</sub> toxicity (Annis, 1987; Bartosik et al., 2001). Experiments with maize (Casini et al. 2009; Rodriguez et al., 2002a), wheat (Rodriguez et al., 2002b), sunflower (Rodriguez et al., 2002c) and soybean (Rodriguez et al., 2002d) determined that a moderate change in the interstitial atmosphere (CO<sub>2</sub> < 10% and O<sub>2</sub> > 10%) usually allows the survival of adults between 60 and 80 days (when you create an atmosphere rich in CO<sub>2</sub> (at least 15%) and low amount of O<sub>2</sub> (< 5%) allows complete control of adult insects in less than a month of storage). However, a severe change in the interstitial atmosphere was associated with a decrease in grain quality parameters (germination test, test weight in wheat, oil acidity in sunflower, etc.) caused by an intense biological activity (mainly due to the activity of fungi). Because of this, good storage practices are designed to minimize the change in atmosphere within the silobag (e.g. storage of grain moisture below the marketing limit), thus relegating the deleterious effect a modified atmosphere in insects.

Besides the atmosphere composition inside the silobag, the grain temperature in the cold season of the year can be a limitation for the development of insects. The average temperature of the grain in the bag varie according to the mean monthly temperature (Bartosik et al., 2009). This means that in temperate or cold temperatures the grain temperature is below 15°C (Cardoso et al., 2009). Under these conditions, the development of insects is slow (Burges and Burrell, 1964) or null (Banks and Fields, 1995). However, the low temperature is not sufficient to achieve complete mortality. From mid-spring, summer and early fall, the temperature of the grain would not be an obstacle for the development of insects.

### 1.3 Alternatives for insect control in silobags

Based on the foregoing, there are favorable situations in which insects can be bagged along with the grain (usually when the grain comes from other storage facilities and to a lesser extent, the insects coming with the grain from the field) and survive during storage (insufficiently modified interstitial atmosphere, storage for short periods of time). While the direct damage caused by insects in the grain may be insignificant, the presence of live insects is a major constraint for

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marketing the grain. According to the grain marketing regulations in Argentina, each load of grain containing live insects must be rejected (FAO, 2009).

Darby and Caddick (2007) argued that a silobag properly filled and maintained can reach a high level of gas tightness. On this basis, the use of fumigants such the phosphine can be a suitable alternative for insect control in airtight plastic bags. Considered as one of the best fumigants today (Cao and Wan, 2001), the phosphine has low cost, is easy to use, leaves no residue (Collins, et al., 2001) and do not affect seed germination (Bond, 2007).

Formulations of aluminum or magnesium phosphide fumigant in the form of pellets or tablets are placed in the grain, which reacts with water in the air to produce a gas fumigant, phosphine. The number of days required for the gas depends on temperature, humidity, brand fumigant (Banks, 1991), specie, strain and poblacion of insect (Anon.,1998; Daghli et al., 2002). It is a slow-acting poison that is effective in very low concentrations, if the exposure time is sufficient. Usually a minimum of four or more days of exposure are needed to control insects (Bond, 2007). Navarro and Noyes (2002) quote the importance that the gas concentration must be maintained above 150-200 ppm during 120 hours to achieve an ideal lethal effect on all insects species.

#### **1.4 General objective:**

Determine a rational methodology of fumigation with phosphine for insect control in grain stored in airtight plastic bags.

#### **1.5 Specific objectives:**

Determine the amount of phosphine tablets to achieve an adequate concentration of gas effective for insect control (200 ppm) throughout the recommended exposure time (5 days).

Determine an appropriate methodology for the placement of the tablets in the mass of grains to reach the effective concentration of the gas quickly and evenly throughout the bag.

Study the dynamics of the fumigant gas concentration during treatment (the delay in achieving the appropriate concentration and how it decays in time).

Determine the level of insect control (mortality).

## **2. MATERIALS AND METHODS**

The test was conducted on the farm “The Monerío”, in the district of Balcarce (Buenos Aires, Argentina) during February 2007.

For this test three silobags were filled with 200 tonnes of wheat harvested during december 2006 at similar moisture contents (between 12,8 and 13,4%). The low moisture content of grain within the silobags ensured a low level of CO<sub>2</sub> in the bag, thus avoiding the concentration of CO<sub>2</sub> has an effect on mortality of the insects by it self. Two silobags were used for fumigation treatments (3

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gr/m<sup>3</sup> aluminum phosphide and 6 gr/m<sup>3</sup> aluminum phosphide), while the third one was used as control (without application of aluminum phosphide).

## 2.1 Calculation of the dose of application

Considering a grain test weight of 750 kg/m<sup>3</sup>, the total estimated volume of the bag was 266 m<sup>3</sup> (200 t / 0,75 t/m<sup>3</sup>).

### 2.1.1 Lower dose (A)

1 tablet/m<sup>3</sup> (3 gr/m<sup>3</sup> of aluminum phosphide tablets or 1 g/m<sup>3</sup> phosphine).

Number of tablets = 266 (798 gr).

### 2.1.2 Higher dose (B)

2 tablets/m<sup>3</sup> (6 gr/m<sup>3</sup> of aluminum phosphide tablets or 2 g/m<sup>3</sup> phosphine).

Number of tablets = 532 (1596 gr).

## 2.2 Mode of application

It was decided to make an application every 5 m along the bag, implying that the phosphine gas should move 2,5 m to each side of the application point. In each application point, the amount of tablets inserted were related to the volume of grain that represents the distance between two applications points. The total length of the bag was 60 m, so 12 application points were identified in the total length of the bag. The first application point was located at 2,5 m from the beginning of the bag (Figure 2).

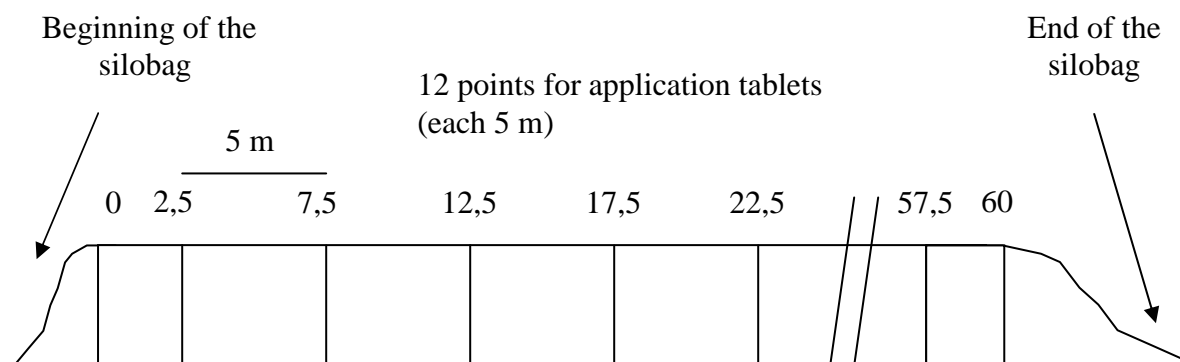


Figure 2. Diagram of side view of the silobag, detailing the placement of aluminum phosphide tablets.

The dose used in each application point was calculated as follows:

A. (lower dose) - 266 tablets in 60 m - Number of tablets per application point =  
 $266 / 12 = 22$  tablets.

B. (higher dose) - 532 tablets in 60 m - Number of tablets per application point =  
 $532 / 12 = 44$  tablets.

The tablets were placed inside the silobag with a plastic tube of 40 mm in diameter. The plastic tube was introduced into diagonally toward the center and bottom of the grain mass. After inserting the plastic tube the tablets corresponding to the application points were introduced in the tube, tacking the precaution of spreading the tablets throughout the profile of the mass of grains in each application point. A violent exothermic reaction could occur if all the phosphine tablets are placed together, in contact between them. This was achieved by lifting of the plastic tube after dropping a few tablets (about 5 tablets), allowing this group of tablets to be covered with grain. The contact between the phosphine tablets and the plastic cover was also prevented by layer of grain to prevent damage of the plastic during the exothermic reaction of the tablets when releasing the gas.

### 2.3 Measurement of the concentration of fumigant

The measurement of gas concentration were conducted with a hand pump and glass ampoules with sensitive material to the fumigant gas (colorimetric tubes). The gas concentration is measured as a change of color (from white to dark brown) on a scale in ppm (Figure 3). The measurement was performed daily from the time of application until the gas concentration dropped below 150 ppm.



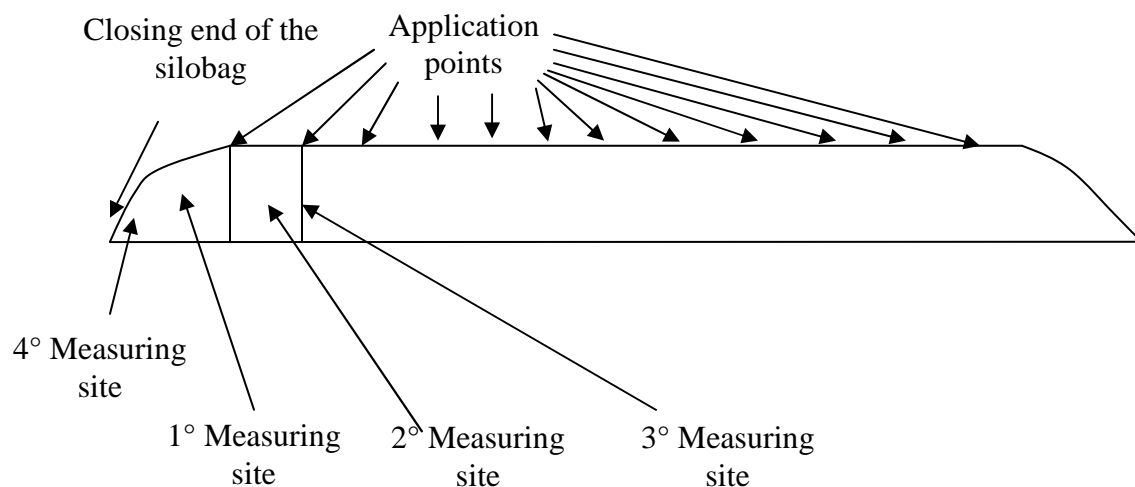
Figure 3. Detail of colorimetric tube showing a concentration of 200 ppm phosphine.

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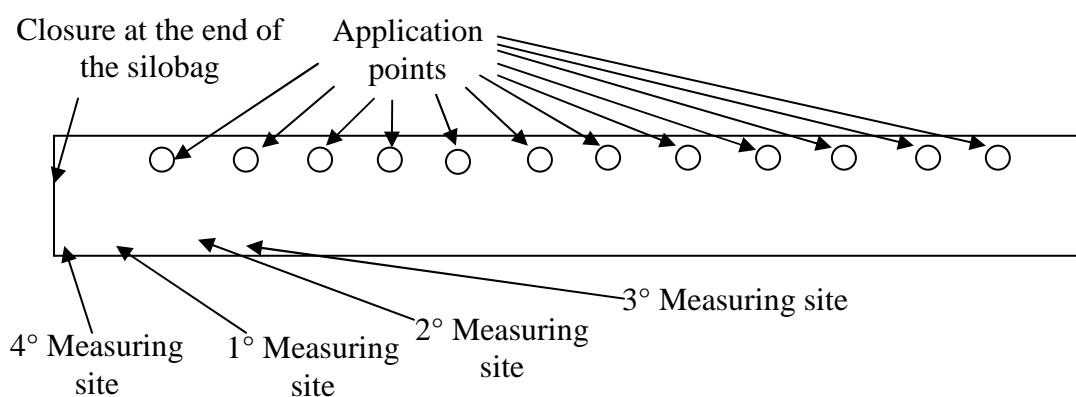
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The fumigant concentration monitoring was focused at the end of the silobag. This sector is the most critical in maintaining the concentration of the gas, due to poor techniques for closing and sealing of the bag. The other end, the beginning of silobag, usually presents fewer problems in maintaining the tightness, since the closure is tightened by the same mass of grains. The monitoring of gas was also performed on the opposite side of the bag where tablets were inserted, to ensure that the phosphine concentration reached the desired level in the farthest point from the application site. The concentration of phosphine was measured in four different places in each treated silobag (Figure 4):

1. Near the closing end of the bag.
2. Between the first two application points (near end of the bag).
3. On an application point.
4. On the closing end of the bag.



A. Side view of the distribution of the application points and measurement sites.



B. Top view of the distribution of the application points and measurement sites.

Figure 4. Diagram of application points of tablets of aluminum phosphide and measurement sites of gas concentration, in lateral view (A) and top view (B).

Moreover, in the silobag treated with higher dose, was replicated phosphine measurements in side application of the phosphide tablets.

Simultaneously with measurements of concentrations of phosphine were measured for CO<sub>2</sub> (Check Point, Dan Sensor, Denmark) concentration and temperature of the grain mass during the test.



## 2.4 Mortality of Insects

To evaluate the effect of the concentration of phosphine on insects, were introduced in the bags cells with insects lives. After completion of the test, were withdrawn the cells to determine the level of mortality or control. To be certain that the mortality of insects was attributed to the effect of the phosphine rather than the concentration of CO<sub>2</sub>, also was repeated the same experiment in the bag control.

The day before test, was filled the cells with live insects. Each cell was armed with a plastic tube of approximately 150 mm in length. The ends of the tube were covered with a fine mesh fabric (Figure 5). Each tubes is filled with wheat and contains 10 weevils (*Sitophilus oryzae*).



Figure 5. Tubes filled with wheat with 10 live insects.

Three of these tubes were placed in a slightly larger diameter pipe (Figure 6). This was completely perforated to allow the passage of gas into the interior of the tubes containing the insects. The big pipes (containing tubes with live insects) were placed in plastic bags in the same spots where the measurement of phosphine (Figure 4). The insertion of the pipe is made from the upper side of the bag diagonally to the area lower central. Plastic tubes containing three insect cages each (10 adults weevils per cage) were inserted in the grain mass. In the places indicated in Figure 6 the plastic tubes presented holes to allow the penetration of the fumigant gas. After the fumigant was performed, the tubes were removed and insect mortality counted. Then, the grain of the cages was placed in incubation at 30°C during 1 month to detect any insect eclotion from eggs.



Figure 6: Details of pipe containing the cages with insects.

### 3. RESULTS AND DISCUSSION

#### 3.1 Phosphine concentration

Figure 7 shows the phosphine concentration reached in the silobag treated with the highest dose ( $6 \text{ gr/m}^3$  aluminum phosphide), since the beginning of the experiment (January 31) until 10 days later. It was generally observed that the peak concentration occurs after one or two days since application. The phosphine concentration in the application side exceeded 200 ppm for 6 days (reaching a peak of 450 ppm on February 2) when was measured on an application point, while between two application points the phosphine concentration was maintained above 200 ppm only during 4 days. Additionally, the objective concentration and exposition time was not achieved at the end of the bag. The phosphine concentration reaches 200 ppm by only one or two days, and this combination of concentration and exposure time is not sufficient to achieve control of insect eggs. As expected, measurement of the fumigant concentration on the same side of silobag where the tablets were inserted was higher and remained above 200 ppm over a longer period of time than in the opposite side of the bag. In the same figure (Figure 7) can be seen that after 7 days from beginning of the experiment, the concentration of phosphine at all measurement points was between 400 and 450 ppm (even in the measurements near the end of the bag). At the end of the measurement period (February 10) the concentration of phosphine was between 200 and 250 ppm.

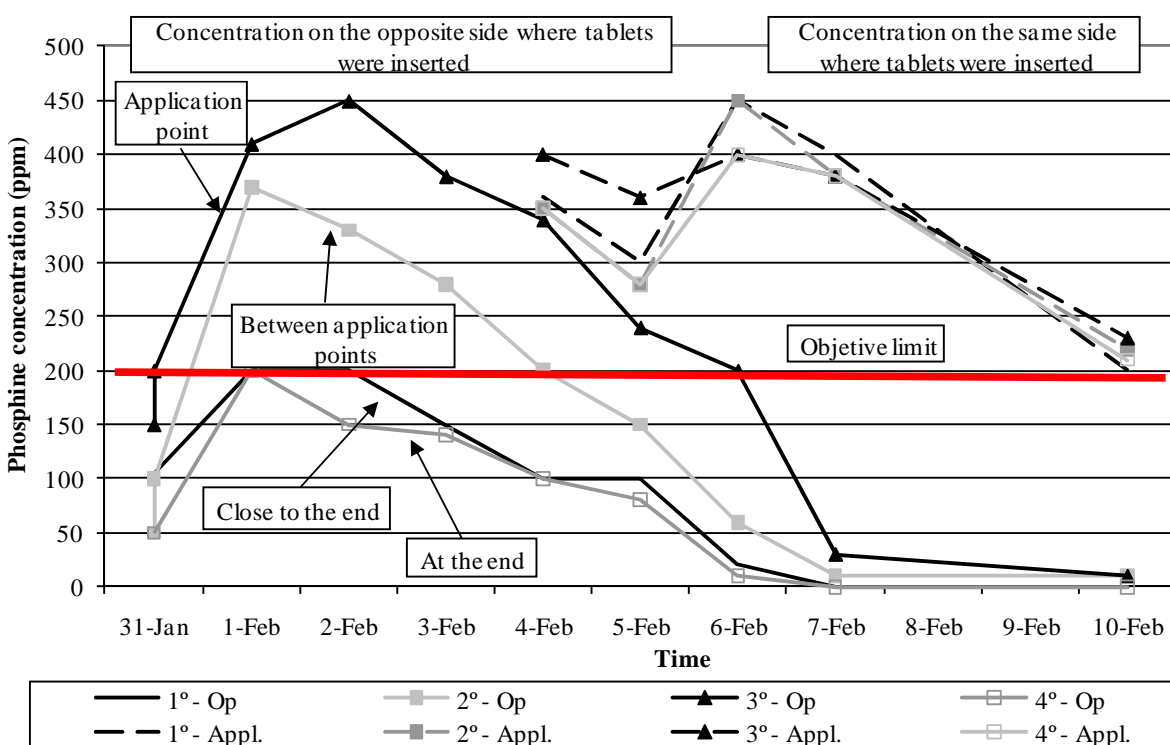


Figure 7: Change in the concentration of phosphine in different measuring points in the bag over time. Treatment: 6 gr of aluminum phosphide/m<sup>3</sup>. References: 1 = near the application point, 2 = between application points, 3 = close to the end of the bag, 4 = at the end of the bag; Op. = measurement on the opposite side where tablets were inserted; Appl. = measurement on the same side where tablets were inserted.

Figure 8 shows the change of phosphine concentration in the silobag treated with the lower dose of the fumigant (3 gr/m<sup>3</sup> aluminum phosphide). This figure, only shows the measured concentration on the opposite side where tablets were inserted. Like what was observed in the experiment with higher doses, the highest concentration was observed after three days from application. The higher concentration of phosphine was observed near the application point (430 ppm), and the concentration of phosphine remained above 200 ppm for 6 days (from February 1 to February 7). In between two application points, the phosphine concentration was above of 200 ppm for 5 days (February 1 to February 6). As in the experiment of higher dose, phosphine concentration near the end of the bag was substantially lower than in the rest of silobag (reaching a peak of 150 ppm on the third day, dropping below 100 ppm on the seventh day).

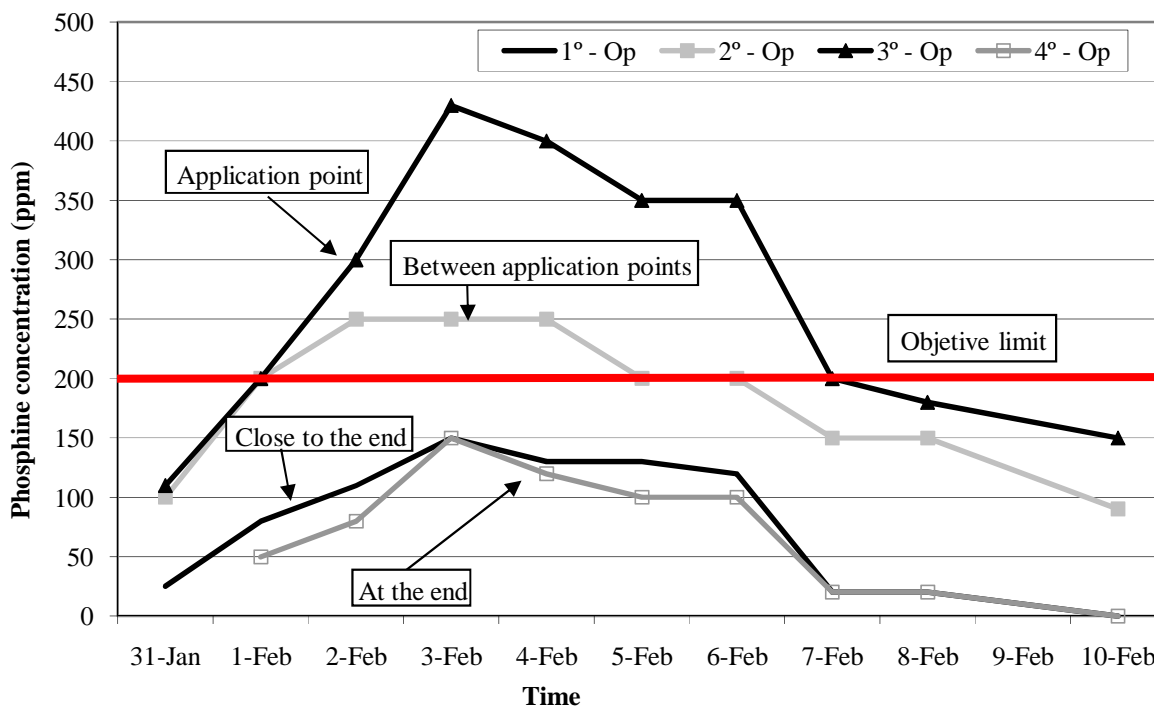


Figure 8: Change in the phosphine concentration in different measuring points in the bag over time. Treatment: 3 gr of aluminum phosphide/m<sup>3</sup>. References: 1 = near the application point, 2 = between application points, 3 = close to the end of the bag, 4 = at the end of the bag; Op. = measurement on the opposite side where tablets were inserted.

Reed and Pan (2000) studied the dynamics of phosphine in sealed bins (filled of wheat and empty) and unsealed (filled of wheat) for different conditions of humidity and temperature of the grain. Under conditions similar to those evaluated in this trial (13,2% moisture and 25°C), the maximum concentration of phosphine in sealed bin was less than 70% of the potential (or PD = potential theoretical dose of phosphine applied; 1 g/m<sup>3</sup> of phosphine applied equals to 718 ppm), whereas in grain without bin were above 90%. This indicates that a significant proportion of the phosphine is adsorbed by grain. In unsealed bin, a concentration of 10-12% PD was achieved. The treatment of higher dose (B) showed a similar evolution of the gas to the sealed bin (maximum phosphine to 1-2 days). However, the maximum levels of phosphine achieved are low compared to the PD, both in the location of the application point (31% of 1436 ppm applied (2 gr/m<sup>3</sup>)) between two points (26% PD), with a continuous decrease in the levels of phosphine in the following days. On the other hand, the maximum levels of phosphine measured near the end of the bag were 14% PD, similar in value that thus for unsealed bin. This indicated that the closure of the bag did not have a high level of tightness, which is clearly shown by the low levels of phosphine obtained and in the relatively rapid decrease of concentration.

In the treatment of lower dose (A) the maximum concentration in the application point (60% PD) and to a lesser degree between application points (35% PD), are significantly better. This implies, a higher level of tightness in the closure of the bag (20% PD). In all measured areas the rate of fall of phosphine was more attenuated in time. This indicates that overall the bag A presented a better tightness than the bag B.

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As shown in Figure 7, the release of most of the phosphine only was delayed a few days. The high rate of release of phosphine allowed a large differences concentration to few meters distance (about 4 meters from the application point until the average distance between application points of the other application side), producing a rapid spread initial. However, there is a gap between the maximum phosphine (and subsequent fall) of the point farthest from the application (2 days) and the maximum phosphine (and subsequent fall) at the site of phosphine application (5 days). This suggests that treatment duration should be at least 10 days of duration (5 to achieve the maximum concentration in all sectors and then 5 days more to expose a concentration of 200 ppm or greater).

The differences in phosphine concentration reached close to the point application and in the application points (both on the opposite side of the application of phosphine), are great in the bag A (180 ppm). This may indicate that in a bag with good degree of tightness, the rate of diffusion of gas is shown as a limiting factor for achieving a homogeneous PD gas throughout the bag. This difference of concentration could be even higher, using a more distant distribution of application points.

From figures 7 and 8 it was observed that most of grains mass received the objective dose (5 days to 200 ppm or more), in both silobags. It indicates the importance of having a higher degree of tightness in the bag, paying close attention to the closing of the bag. This is visualized on the differences between the two silobags tightness, especially in poor sealing of the closure system, causing leakage of the fumigant product. As in this case, usually the bags are closed by folding the plastic over, covering the end with dirt or any heavy object. A more efficient methodology for closing the bag (the thermo-sealing, or a closure between two slats of wood nailed and coil) is recommended. In addition to performing an adequate job in closing the bag, it is also recommended increasing the dose in the area close to the end to compensate for any possible loss of tightness (e.g. implement the double or triple the concentration of tablets in the last 4 m bag). Also, a replication of the dosage at the closure of the bag after 3 days would help to maintain an effective concentration.

### 3.2 Mortality of Insects

The CO<sub>2</sub> concentration of the silobags during the treatments was below 3,5% (average 1,5% in the bag A, 2,5% in the bag B and the bag control) insufficient to kill insects by itself.

Table 1 shows the results of the effect of different treatments with aluminum phosphide (3 and 6 gr/m<sup>3</sup>) and in at the control treatment on the mortality of adult insects. The study found that even when at the closing ends of the bag did not reach the prescribed concentration (200 ppm) during the desired exposure time (5 days), it was enough to control 100% of adults, even in the treatment of lower dose. In turn, the control silobag, showed a percentage of mortality in the range of 13 to 33%. This mortality may be due to unfavorable storage conditions, characterized by low relative humidity (low humidity of the grain in the bag) and high temperature fluctuation of the grain mass, at the least in the top layers. Evans (1983) shows the combined effect of temperature and relative humidity on the survival of adult *S. oryzae*. Exposed at relative humidity of 70 and 45% and temperature of 30°C, the lifetime decreases from 30 to 19 weeks, but when the temperature is 13°C shows no differences. Rodriguez et al. (2002b) under similar

conditions (12,5% moisture and high temperature of the grain), observed total mortality of adult insects of weevils before 45 days.

The lethal effect of low levels of phosphine in the closing end of silobag, would indicate that the degree of resistance in the insect population to phosphine was not high. Nayak, et al. (2003, 2007) indicate that 200 ppm of phosphine can achieve control populations of susceptible adults in hours and even resistant populations (Australia) in 2 days. When it comes to populations with high resistance (China) requires 16 days of exposure to such concentration.

Although only assessed adult mortality, there are references that say of a control of all stages of *S. oryzae* (including eggs and pupae) when exposed to 200 ppm for 5 days in resistant populations to phosphine. Mills and Athie (2000), achieved similar control with a dose of 350 ppm in just 72 h (temperature of 25°C and 60% relative humidity), which would indicate that with a time of 120 h of exposure a lower dose would be sufficient. To control a resistant population will require or increase the dose to 600-700 ppm for 5 days or maintain the target dose for at least 7 days (Nayak, et al. 2001), concentration that was achieved only in some areas of treatment silobags.

One aspect that could achieve better control the dose of phosphine obtained, relates to the fact that CO<sub>2</sub> could be used as an adjuvant to spray for phosphine (Ren et al., 1994). Athie et al. (1998) reported that CO<sub>2</sub> increases the toxicity in phosphine-resistant strains of several species of beetle. Kashi and Bond (1975) found that the absorption of phosphine and mortality of *Sitophilus granarius* and *Tribolium confusum*, increased by 4% CO<sub>2</sub>. The effect of low doses of CO<sub>2</sub> is by increasing the metabolism of insects. Insect respiration can be increased by 50% by increasing the CO<sub>2</sub> levels to 3% or to 300% when CO<sub>2</sub> levels are raised to 5% (Mueller, 1994). This would indicate that even when the silobags had a low level of CO<sub>2</sub> (3%), which cannot cause mortality by itself, this low concentration of the gas could potentiate the killing effect of the phosphine. This situation implies that fumigating grain in hermetic plastic bag would have two potential benefits: 1) take advantage of the airtightness of the system; 2) extra killing effect of the phosphine due to presence of CO<sub>2</sub> in concentration of about 3%.

Table 1. Insects mortality in cells located at different depths (upper, middle and bottom) in three different places of silobag (close to application point, between two application points, and near the end of the bag) in the treatments with 3 and 6 g/m<sup>3</sup> aluminum phosphide and in the control at to 10 days after initiating the experiment.

Treatment	Place	Depths	Repetition			Average (%)
			1(%)	2(%)	3(%)	
Control		Upper	20	10	40	23
		Middle	20	20	60	33
		Bottom	20	20	0	13
3 gr/m <sup>3</sup> *	Application point	Upper	100	100	100	100
		Middle	100	100	100	100
		Bottom	100	100	100	100
	Between application point	Upper	100	100	100	100
		Middle	100	100	100	100
		Bottom	100	100	100	100
	Closure of silobag	Upper	100	100	100	100
		Middle	100	100	100	100
		Bottom	100	100	100	100
6 gr/m <sup>3</sup> *	Application point	Upper	100	100	100	100
		Middle	100	100	100	100
		Bottom	100	100	100	100
	Between application point	Upper	100	100	100	100
		Middle	100	100	100	100
		Bottom	100	100	100	100
	Closure of silobag	Upper	100	100	100	100
		Middle	100	100	100	100
		Bottom	100	100	100	100

\* The concentration of 3 and 6 g/m<sup>3</sup> aluminum phosphide (Phostoxin) correspond to 1 and 2 g/m<sup>3</sup> phosphine, respectively.

#### 4. CONCLUSION

- The phosphine concentration in both experiments reached or exceeded 200 ppm in most parts of the bag during the 5 days required to control most insect pests. Therefore, we can conclude that treatment with 3 gr/m<sup>3</sup> of aluminum phosphide (266 tablets in the entire bag) was sufficient to achieve the intended objective.
- The near area to end of the bag posed problems of gas leak, preventing the phosphine concentration reaches a 200 ppm and maintained at those values for 5 days.
- To guarantee that the phosphine concentration is adequate in the whole bag, is necessary prevent that has zones with leakage of gas, with special care on the closing of the bag. In turn, it would be desirable to increase the amount of product in the end of the bag, as well as advisable make a re-application after 2-3 days of starting treatment.
- The phosphine concentration reached higher values in areas close to the points of application. However, a distribution of the points of application of 5 linear meters along the bag was sufficient, achieving the peak concentration in the far (between two points of

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application and on the opposite side of the bag) at three -four days of starting treatment. The insect control treatment with phosphine is then implemented within a period of not less than 9 days.

- The phosphine concentration decays slowly after the fourth day of application, with values of 150 ppm in some areas of the bag after 10 days of beginning treatment. For these reasons it is important taken precautions to handle of grain in bags that have recently been treated with this product.
- The mortality of adult insect was of 100%, still in the bag treated with the lowest dose (3 gr/m<sup>3</sup> aluminum phosphide), while that the insects mortality in the control silobag (untreated) was only 13 to 33 %.

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