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Physiological and sanitary performance of soybean seeds during storage after phosphine fumigation[1]

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ABSTRACT – To maintain the physical, physiological and sanitary qualities of soybean seeds during storage, efficient control of pests must be considered. Fumigation is a technique used to eliminate pests that infest stored seeds, but there is no information on possible toxic effects on the seed and the microorganisms in the seeds fumigated several times during storage. An experiment was conducted to ascertain its effect on germination, viability, and vigor (tetrazolium, accelerated aging, and seedling length tests) and on the microorganisms associated with the soybean seeds. The study was conducted on soybean seeds which were exposed to three phosphine fumigations over the period of 210 days of storage in a completely randomized statistical design. The treatments were three concentrations of phosphine ($PH_3.m^{-3}$) at 1.0, 2.0 and 3.0 $g.m^{-3}$ and the control without application of phosphine, applied three times on the same group of the seeds throughout the storage period. The fumigation chambers were made of plastic sheet impermeable to phosphine gas. Seed fumigation did not modify the physiological performance of the soybean seeds. It also did not affect the viability of the fungi and bacteria in the seeds.

Index terms: *Glycine max*, pests, germination, vigor, microorganisms.

Desempenho fisiológico e sanitário da semente de soja durante o armazenamento após expurgo com gás fosfina

RESUMO – Para manter as qualidades física, fisiológica e sanitária das sementes de soja durante o armazenamento, deve-se considerar o controle eficiente de pragas. O expurgo é uma técnica usada para eliminar pragas que infestam sementes armazenadas, mas não há informações sobre possíveis efeitos fitotóxicos ou efeitos sobre os microrganismos nas sementes expurgadas várias vezes durante o armazenamento. Um experimento foi conduzido para verificar o efeito na germinação, viabilidade e vigor (tetrazólio, envelhecimento acelerado e testes de comprimento de plântulas) e na viabilidade dos microrganismos associados às sementes de soja. O estudo foi conduzido em sementes de soja que foram expostas a três expurgos com fosfina durante o período de 210 dias de armazenamento, em delineamento estatístico inteiramente casualizado. Os tratamentos foram três concentrações de fosfina (PH_3) $.m^{-3}$ a 1,0, 2,0 e 3,0 $g.m^{-3}$ e o controle, sem aplicação de fosfina, aplicados três vezes nas mesmas sementes ao longo do período de armazenamento. As câmaras de expurgo foram feitas de lona plástica impermeável ao gás fosfina. O expurgo das sementes não modificou o desempenho fisiológico das sementes de soja. Também não afetou a viabilidade dos fungos e das bactérias presentes nas sementes.

Termos para indexação: *Glycine max*, pragas, germinação, vigor, microrganismos.

Introduction

The physiological and sanitary qualities of soybean seeds must be maintained over the storage period so that their use may lead to establishment of crop fields with high yield potential. Pests may affect these qualities during storage and, for that reason, seeds are fumigated and phosphine gas is most used for that purpose. Fumigation is a routine practice in seed warehouses to prevent pest damage.

The main pests that occur in storage of soybean seeds

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are the Coleoptera *Lasioderma serricorne*, *Cryptolestes ferrugineus*, *Oryzaephilus surinamensis*, *Sitophilus oryzae, Rhyzopertha dominica, Tribolium castaneum, Ahasverus advena*, and *Lophocateres pusillus*, and the Lepidoptera *Ephestia elutella, Ephestia cautella*, and *Sitotroga cerealella* (Lorini, 2012; Lorini et al., 2015). *L. serricorne* develops in soybean seeds during the storage period, justifying routine fumigation with phosphine to prevent losses (Ferri et al., 2018).

Seed fumigation with the use of phosphine gas (PH_3) is a technique that has been used for several years on several products, however, only recently in storage of soybean. PH_3 is a general biocide, highly toxic, and released in the presence of humidity in the air; it is effective in controlling all phases (egg, larva, pupa, and adult) of pests in stored seeds (Lorini, 2012; Lorini et al., 2013).The distribution of gas released within the seed mass in storage must be uniform at all points, allowing control of all the pests in the different forms of the life cycle (Lorini et al., 2015). The rate of release of phosphine gas coming from the fumigation tablets will determine the time necessary to reach the minimum effective concentration of 400 ppm for controlling all the pests, which should remain for a minimum of 120 h to ensure efficiency in the fumigation process (Daglish et al., 2002; Lorini et al., 2011; 2015).

With the recent use on soybean seeds and the status of phosphine as a biocide, there are scientific questions regarding its effect on seed physiological quality and the viability of fungi and bacteria when the seed is fumigated several times over the storage period, a frequent practice in the Brazilian seed industry.

The aim of this study was to evaluate the effects of sequential fumigations with different concentrations of phosphine applied during the storage period on the germination and vigor of soybean seeds and on the viability of the fungi and the bacteria in the seeds.

Material and Methods

An experiment was set up at the Technological Center of Seed and Grain Science of Embrapa Soybean in Londrina, PR, Brazil, with two soybean cultivars exposed to three fumigations each at three concentrations of phosphine during the storage period. Soybean seeds with high vigor levels were used: 92% for seeds of the cultivar BRS 232; and 82% for Embrapa 48, as determined by the tetrazolium test. The experiment was conducted in a randomized block design, with four replications. Each experimental unit (replication of each treatment) was composed of 3.0 kg of seeds placed in individual 1.0 m^3 fumigation chambers made of plastic sheet impermeable to phosphine gas. Concentrations of 1.0, 2.0 and 3.0 g of $PH_3.m^{-3}$ were used, and one control treatment without application of phosphine.

The concentration of phosphine gas within each chamber was monitored daily for nine days using the Silochek® gas meter, which is a device that determines the concentration of gas measured in ppm released by commercial tablets of aluminum phosphide. After the period of the first fumigation, at the beginning of the storage period, the fumigation chambers were opened, the seeds were removed, and a sample from each experimental unit was taken for analyses of quality by the tests of germination (Brasil, 2009), seedling length, hypocotyl length, root length, accelerated aging (Krzyzanowski et al., 1999), vigor and viability by the tetrazolium test (França-Neto et al., 1998), and seed health testing through the blotter test (Henning, 2015).

After that, the experimental units of seeds were stored in a room environment in the Seed Processing Unit for a period of 90 days, after which they were exposed to the second fumigation, under the same method described above. They were then stored for 120 days and, finally, the third fumigation was performed. After each phosphine fumigation, a sample was removed from each replication for seed physiological and sanitary analysis.

The physiological quality of the seeds was evaluated by the germination and accelerated aging tests using 200 seeds (four 50-seed subsamples) for each replication. Vigor and viability of the seeds were evaluated by the tetrazolium test, where 100 seeds (two 50-seed subsamples) were used from each experimental unit, and for the seedling length test, 100 seeds (five 20-seed subsamples) each. The blotter test was performed on 200 seeds (ten 20-seed subsamples) for each replication of the experiment.

The results from phosphine concentration during each fumigation were displayed in diagram form, and analysis of variance was conducted on the other data per cultivar and per fumigation time (1st, 2nd and 3rd) to isolate the effects of the differences in vigor levels between the cultivars and in environmental conditions throughout the storage period. The means were separated by the Tukey test at the 5% level of probability using the SASM-Agri statistical software (Canteri et al., 2001).

Results and Discussion

The concentration of phosphine remained well distributed within the chambers in the three fumigations made in the experiment, with the greatest release of gas by the fumigation tablets occurring within the first 48 h. This release decreased over time (up to 216 h), but remained above 400 ppm for the minimum period of 120 h for the concentrations of 2.0 and 3.0 $g.m^{-3}$ of PH_3 (Figures 1 to 3). However, the concentration of 1.0 $g.m^{-3}$ of PH_3 in the first two fumigations (Figures 1 and 2) was below the technical reference effectiveness for fumigation, which must be maintained for a minimum of 120 h at a concentration greater than 400 ppm to eliminate all the life phases of the insect pests of stored seeds (Daglish et al., 2002; Lorini et al., 2015).

In the results of assessment of seed physiological quality, the three concentrations of phosphine applied, i.e., 1.0, 2.0 and 3.0 g.m^{-3}, did not show significant differences in relation to the control without application of the gas (Table 1). In general,

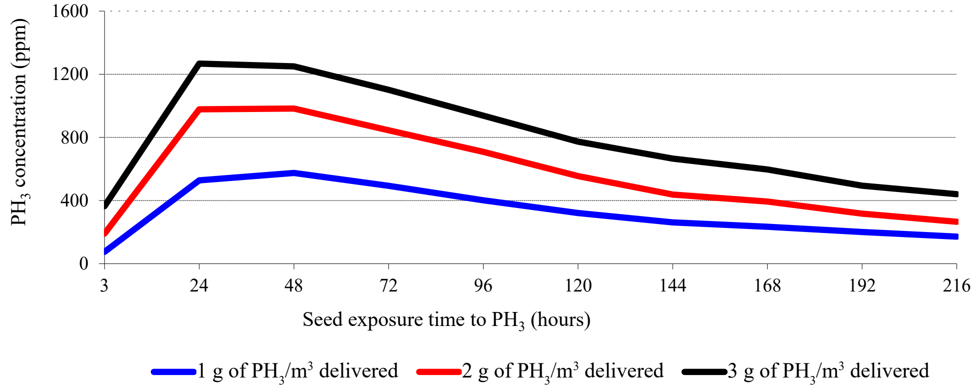

Figure 1. Phosphine (PH_3) concentration during the first fumigation (beginning of storage) of soybean seeds of BRS 232 and Embrapa 48 cultivars.

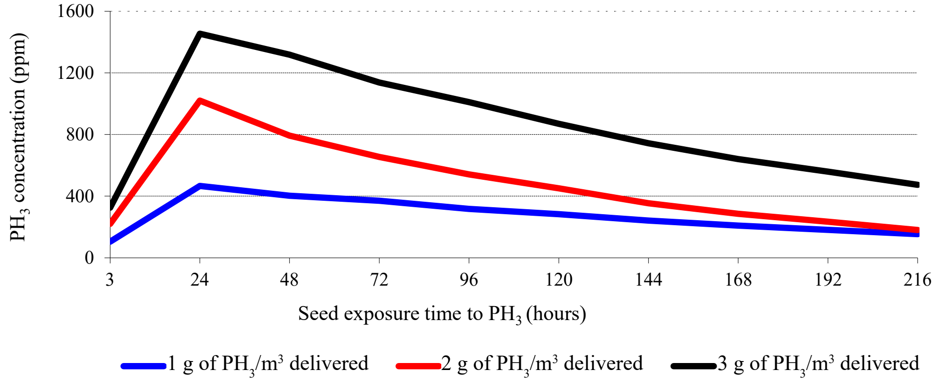

Figure 2. Phosphine (PH_3) concentration during the second fumigation (after 90 days of storage) of soybean seeds of BRS 232 and Embrapa 48 cultivars.

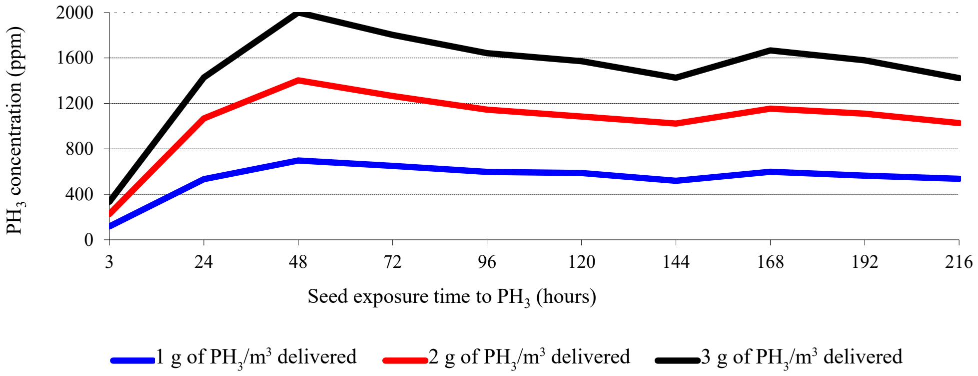

Figure 3. Phosphine (PH_3) concentration during the third fumigation (after 210 days of storage) of soybean seeds of BRS 232 and Embrapa 48 cultivars.

Table 1. Effect of the fumigations at different concentrations of phosphine on soybean seed physiological performance during storage. Embrapa Soybean.

Treatment		Tetrazolium		Accelerated aging (%)	Germination (%)	Seedling length (cm)		
Cultivar	Phosphine concentration (g.m^{-3})	Vigor (%)	Viability (%)			Total length	Hypocotyl length	Root length
First fumigation:								
BRS232	Control	92 [1]	96 [1]	92 b	96 [1]	19.0 [1]	6.0 [1]	16.7 [1]
High Vigor	1.0	90	96	92 b	96	17.9	5.4	12.0
TZ 92%	2.0	91	95	94 a	97	17.5	5.6	11.2
	3.0	93	97	94 a	97	17.9	5.8	11.5
C.V.%		1.72	2.07	0.66	0.87	6.28	7.80	7.12
First fumigation:								
Embrapa 48	Control	82 [1]	90 [1]	83 [1]	90 [1]	24.6 a	7.8 [1]	16.7 a
High Vigor	1.0	81	89	80	90	19.6 b	7.5	12.0 b
TZ 82%	2.0	82	90	83	91	18.7 b	7.4	11.2 b
	3.0	80	88	81	92	19.0 b	7.5	11.5 b
C.V.%		4.24	2.88	2.57	1.67	4.90	3.55	7.27
Second fumigation:								
BRS232	Control	94 [1]	96 [1]	93 [1]	91 [1]	16.5 b	5.4 b	11.0 b
High Vigor	1.0	91	96	91	90	16.3 b	5.6 b	10.7 bc
TZ 92%	2.0	92	95	94	88	17.7 a	5.4 b	12.3 a
	3.0	93	98	94	87	16.2 b	6.4 a	9.7 c
C.V.%		1.79	1.65	1.89	2.53	3.17	8.21	4.72
Second fumigation:								
Embrapa 48	Control	76 [1]	89 [1]	70 [1]	87 [1]	26.9 a	8.3 a	18.5 a
High Vigor	1.0	75	87	72	91	23.2 b	7.9 ab	15.3 b
TZ 82%	2.0	75	88	76	90	18.8 d	7.4 b	11.4 d
	3.0	76	89	73	88	21.8 c	8.6 a	13.1 c
C.V.%		4.00	2.52	5.26	2.53	3.87	6.14	4.78
Third fumigation:								
BRS232	Control	87 [1]	93 b	89 [1]	93 [1]	15.1 [1]	5.0 [1]	10.0 [1]
High Vigor	1.0	85	93 b	87	94	15.8	5.2	10.6
TZ 92%	2.0	91	96 a	94	94	15.6	5.0	10.5
	3.0	88	93 b	90	93	16.1	5.1	10.9
C.V.%		2.58	1.73	3.23	1.78	4.82	7.92	6.39
Third fumigation:								
Embrapa 48	Control	61 [1]	84 [1]	40 b	76 [1]	18.3 a	7.1 a	11.2 a
High Vigor	1.0	65	83	54 a	82	14.9 b	6.0 b	8.9 b
TZ 82%	2.0	56	82	53 a	83	15.3 b	6.2 b	9.05 b
	3.0	60	83	48 a	82	16.8 ab	6.7 a	10.1 ab
C.V.%		14.56	4.20	9.42	3.86	5.96	3.35	9.14

Means followed by the same letter in the column within the same fumigation and same cultivar are not different from each other by the Tukey test at 5% probability.
[1]Not significant.

the application of phosphine through fumigation of seeds did not affect seed germination and vigor. The seedling length test, with prominence of root length, detected reductions in seedling length of the cultivar Embrapa 48 at the three concentrations of phosphine applied in the three fumigations evaluated, in relation to the control. In the cultivar BRS 232, only variation in root length after the second fumigation was observed. These variations observed in the two cultivars, though statistically significant, do not compromise seed physiological performance since they are low amplitude numerical distances, that were detected because the low coefficient of variation. Krzyzanowski et al. (2013) found similar results, when they evaluated a single application of phosphine on soybean seeds for an exposure period of only 168 h; they found no negative effect of the phosphine fumigation.

Andrade and Nascimento (1984), working with maize and sorghum seeds, and Rocha-Junior and Usberti (2007), working with seeds of wheat cultivars, also did not observe immediate effects from phosphine fumigation on seed physiological performance, corroborating the results obtained here.

The main fungus present in the seeds was *Cercospora kikuchii*, the causal agent of purple seed stain, which occurred at levels of up to 5.1% on BRS 232 seeds; however, the fungus gradually lost its viability over the storage period in all the experimental units, with or without phosphine fumigation (Table 2). The natural presence of saprophytic bacteria was relatively high in seeds of the cultivar Embrapa 48; however, no effect of phosphine was observed on viability of the bacteria, which are normally associated with non-viable seeds (Table 2).

Table 2. Ocurrence of *Cercospora kikuchii* and saprofitic bacteria in soybean seeds, after fumigation with different concentrations of phosphine (PH_3). Embrapa Soybean.

Concentration of phosphine applied (g.m-^{3})	Fumigation applications on seeds during storage		
	First (day one)	Second (90 days)	Third (210 days)
Seeds of BRS 232 infected with *Cercospora kikuchii* (%):			
Control	5.10	1.00	0.00
1.0	1.90	1.25	0.00
2.0	3.90	1.25	0.00
3.0	5.10	1.25	0.00
Seeds of Embrapa 48 with saprofitic bacteria (%):			
Control	15.10	7.90	15.40
1.0	11.50	12.30	12.30
2.0	7.90	12.00	10.60
3.0	7.30	5.90	26.60

Conclusions

The fumigation of seeds with phosphine three times, at concentrations from 1.0 to 3.0 g.m^{-3}, applied over the storage period of 210 days, did not modify the physiological performance of the soybean seeds. It also did not interfere in the viability of the fungi and bacteria in the seeds.

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