A small portion of soaked grain (0.4% d.m.) could be used as O_2 depletor to create an effective modified atmosphere during storage of dry products in hermetic systems made of liners without O_2 barriers or with small perforations.

This is a simple and inexpensive approach to reduce food losses under low cost hermetic storage systems.

O_2 depletors made of chemical compounds could be investigated to obtain the same results as using soaked grain, but without generating unpleasant smell.

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References

ABALONE, R., GASTÓN, A., BARTOSIK, R., CARDOSO, L. AND J. RODRÍGUEZ, 2011a: Gas concentration in the interstitial atmosphere of a wheat silo-bag. Part II: Model sensitivity and effect of grain storage conditions. Journal of Stored Product Research. 47, 276–283.

ABALONE, R., GASTÓN, A., BARTOSIK, R., CARDOSO, L. AND J. RODRÍGUEZ, 2011b: Gas concentration in the interstitial atmosphere of a wheat silo-bag. Part I: Model development and validation. Journal of Stored Product Research. 47, 268–275.

ASAE, 2007: ASAE D245.6 OCT2007 - Moisture relationships of plant-based agricultural products.

CARDOSO, L., BARTOSIK, R., CAMPABADAL, C. AND D. DE LA TORRE, 2012: Air-Tightness Level in Hermetic Plastic Bags (Silo-Bags) for Different Storage Conditions, in: Navarro, S., Banks, H.J., Jayas, D.S., Bell, C.H., Noyes, R.T., Ferizli, A.G., Emekci, M., Isikber, A.A., and K..Alagusundaram, [Eds.], Proceedings of the 9th International Conference Controlled Atmospheres and Fumigation of Stored Products, October 15 to 19 of 2012, Antalya, Turkey..583–589.

CARDOSO, L., BARTOSIK, R., CASTELLARI, C., ABADÍA, B., DE LA TORRE, D. AND H. TAHER, 2016: Hermetic storage of wet corn in liners with and without EVOH barrier., in: Navarro, S., Jayas, D. and K. Alagusundaram (Eds.), Proceedings of the 10th International Conference of Controlled Atmospheres and Fumigation of Stored Products. CAF Permanent Committee, New Delhi, India, November 6-11 of 2016, pp. 117–128.

CARPANETO, B., BARTOSIK, R., CARDOSO, L. AND P. MANETTI, 2016: Pest control treatments with phosphine and controlled atmospheres in silo bags with different airtightness conditions. Journal of Stored Product Research. Submitted, 143–151.

NAVARRO, S., 2012: The use of modified and controlled atmospheres for the disinfestation of stored products. Journal of Pest Science. (2004). 85, 301–322.

NAVARRO, S., 1998: Pressure tests for gaseous applications in sealed storages: theory and practice, in: Zuxun, J., Quan, L., Yongsheng, L., Xianchang, T., and Lianghua, G. (Ed.), Proceedings of the 7th International Working Conference on Stored-Product Protection. IWCSPP, Beijing, China, pp. 385–390.

NAVARRO, S., TIMLICK, B., DEMIANYK, C.J. AND N.D.G. WHITE, 2012: Controlled or Modified Atmospheres, in: Hagstrum, D., Phillips, T., Cuperus, G. (Eds.), Stored Product Protection. Kansas State University, Manhattan, Kansas, USA, pp. 191–202.

OCHANDIO, D., BARTOSIK, R., GASTÓN, A., ABALONE, R., ARIAS BARRETO, A. AND A. YOMMI, 2017: Respiration rate of soybean seeds (Glycine max) in hermetic storage. Enviado para su revisión a J. Stored Prod. Res.

Biocidal efficacy of nitrogen (anoxic atmosphere) applied in operational condition to stored hazelnuts against pest insects at different stages of development.

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Abstract

Recently, a test was conducted in Italy for the evaluation of the biocidal efficacy of Nitrogen saturation (anoxic conditions). One application was carried out in a controlled atmosphere cell of a logistic center specialized in receiving, storing and shipping foodstuffs. The cell, circa 3682 m^3 volume, with capacity of 752 big bags of fresh shelled hazelnuts on 4 height levels was saturated with Nitrogen (99,9%) and maintained at 15-18°C for 21 days. Five test species of insects *Plodia interpunctella*, *Cadra cautella*, *Corcyra cephalonica*, *Tribolium confusum*, *Oryzaephilus surinamensis* were observed at different development stages (egg, larva, adult). The target species were sorted in special biotest and inserted in the big bags to simulate an infestation. At the end of the exposure period the biotests were collected and analyzed. The treatment resulted sufficient to achieve a total control on eggs of Lepidoptera test species only. This result confirmed and integrates the available information in literature that showed the need of a longer minimum exposure period for total control of common stored pest insects.

Keywords: stored pest insects, nitrogen, anoxic atmosphere, entomological biotest.

Introduction

As a result of the ban to use of the methyl bromide and the necessity to use control techniques with minimum impact on men and the environment, the attention was increased in treatments that do not involve the use of biocidal molecules (Navarro, 2006; Fields, *et al.,* 2002; Fleurat-lessard, 1990). In recent years new technologies have been developed to increase the efficiency and effectiveness of physical control methods such as modified atmospheres (Conyers and Bell, 2004). Modified or controlled anoxic atmospheres, including nitrogen, are among the most promising non-toxic alternatives for control of stored product insects and mites in many types of dry stored products (Aulicky *et al.,* 2016). The same authors also reported that ten days of exposure to a concentration of 99% N_2 led to 100% mortality of all adults of *Tribolium castaneum* (Herbst) and *Sitophilus granarius* (L.) at two different level in a metal silo bin. A study in grain at less than 12% moisture, 23°C with 98-100% N_2 concentration, showed that 28 days were needed to kill all the insect pests; while to reach the same insecticidal effect at 18°C the treatment lasted 105 days (Jian *et al.,* 2016).

The present work is aimed to providing data support to avoid phosphine in the process of stocking fresh hazelnuts, verifing that the biocidal effect of the exposure to 99.9% concentration of N_2 for 21 days at 15-18°C temperature is sufficient to ensure total control on the common pests of stored food, in particular of shelled hazelnuts, by evaluation on alive insects at different stages of development immersed within special probes, here named biotest, in 58 big bags that were homogeneously sorted in the cell space.

Materials and Methods

Insects

The insects used as test species were provided by Agroblu Laboratory of Applied Entomology (LEAA), where they were raised at 26 ± 2 °C, 70% RH and photoperiod light-darkness 16:8.

The test organisms used were typical insects infesting hazelnuts such as Plodia interpunctella (Hübner), Cadra cautella (Walker), Corcyra cephalonica (Stainton), Tribolium confusum (Jaqcquelin du Val) and Oryzaephilus surinamensis (Linnaeus) at different stages of development (egg, larva, adult).

Per each of the 58 test unit (big bag), one group of 7 Biotest was prepared and provided (table 1).

Tab. 1 Species, stages and substrates used for the test.

Insect	Stage	Quantity	Substrate
P. interpunctella	Eggs	50	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran
P. interpunctella	Larvae	10	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran
C. cautella	Eggs	50	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran
C. cautella	Larvae	10	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran
C. cephalonica	Eggs	50	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran
T. confusum	Mix population	20 eggs 5 larvae 5 adult	Semolino, brewer's yeast, bran
O. surinamensis	Mix population	20 eggs 5 larvae 5 adult	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran

Substrates

Biotests containing adult insects were prepared with non-infested substrate normally used for breeding. In order to ensure the presence of all insects stages at the same time in biotest containing *Coleoptera,* 10 adults were transferred from the breeding to 10 grams of uninfested substrate 21 days before introduction in the big bags.

Insects were prepared in containers suitable for being inserted into big bags. At the same time, control biotests were prepared and transported with all the others but were kept away from the treatment in order to verify possible mortality during transport. An equal number of control biotest were left at LEAA at 26 ± 2 ° C; 70% HR; L: B = 16: 8.

Test site data

The site selected for the test was a logistic center with climatic cell. The cell has a controlled atmosphere permanent plant, capable of extracting oxygen and pumping nitrogen to reach 99.9% saturation of nitrogen at a chosen range of temperature (15-18°C). Such range of temperature was chosen to achieve a minimum pest development condition. This cell has 752 pallets capacity, sorted in 9 lines of 8 units, each replicated by 4 vertical levels (Fig. 1). The total dimension of the cell is 27.8 m length, 15.14 m width and 8.60 m height.

Fig. 1 The cell structure.

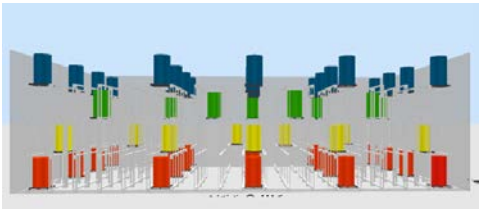

Fig. 2 The 58 big bags sorted in all cell levels from side 1

Test System and application

The test system is characterized by 58 fresh shelled hazelnuts big bags. Each big bag was considered as test unit and was sorted in a specific position in the cell to ensure homogeneus distribution in the cell at all 4 levels. (Fig. 2 and 3).

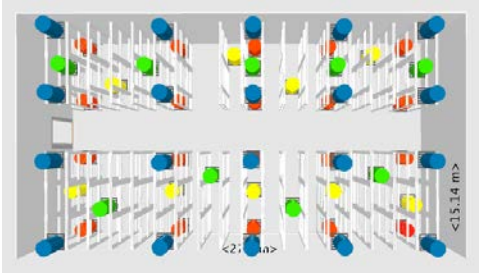

Fig. 3 The 58 big bags sorted in all cell levels from top

Fig. 4 Average temperature registered during the test in the treatment chamber

The test started when the N_2 saturation reached 99.9%. After 21 days of complete and constant N_2 saturation, the desaturation process started.

At the end of de desaturation process LEAA staff could remove all the biotests from the 58 big bags by pulling the relevant twines, then packing and transporting the biotests back to LEAA in the same day.

Table 2 shows the cronological details of the test.

Tab. 2 Test phases

Activities	Progress
Introduction of biotests	Day 1
Saturation process	Day 2
Complete saturation	Day 5
Desaturation process	Day 21
Complete oxygenation	Day 24
Biotest evaluation	Day 25-30

Evaluation method

All the evaluations of the biotests were performed at LEAA by the laboratory staff, through visual assessment, count and record of alive and dead individuals, within 5 days after the extraction.

The assessment was based on the observation of alive individuals. The assessment was recorded as "positive" at the first alive individual observed.

Climatic data and atmosphere monitoring

The internal temperature of the big bags during the trial were collected at least every 60 minutes by digital data loggers provided by the laboratory and immersed together with the biotests in 10 of the 58 test units. The graph (Fig. 4) below represents the mean temperature during the application period (average of ten big bags).

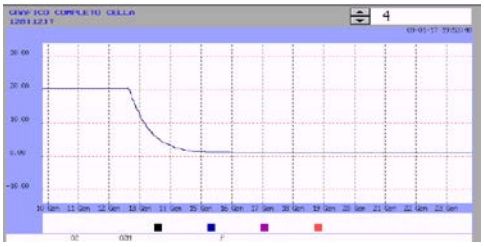

Fig. 5 Percentage of oxygen during the process

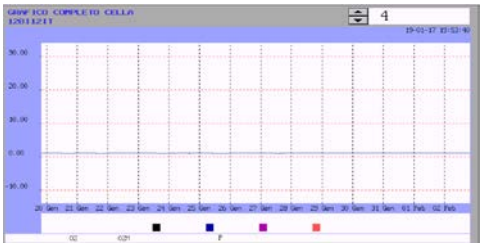

Fig. 6 Percentage of oxygen during the process

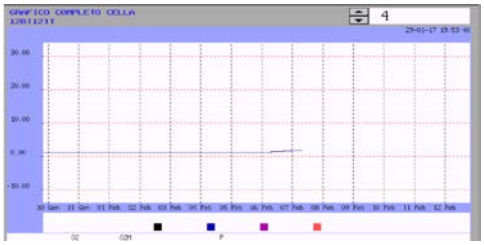

Fig. 7 Percentage of oxygen during the process

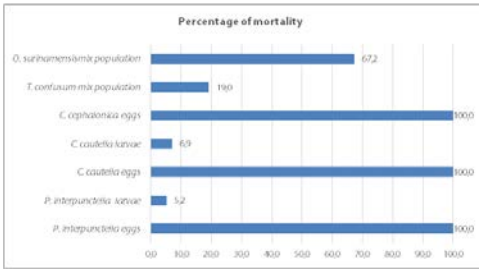

Fig. 8 Percentage of mortality of the different insect species and stages

During the application, the oxygen % in the cell was constantly monitored by a built-in oxygen meter. The data wasprovided by the storage company and reported in the figures 5, 6 and 7.

Results

The results elaborated with Abbott's formula showed that the high effect of the treatment was reached on the eggs of the lepidoptera species (100% mortality). On the Coleoptera species the mortality observed was 19.0% and 67.2% respectively for *T. confusum* and *O. surinamensis* (Fig. 8).

Tables 3 and 4 highlights the percentage of mortality and the number of biotest containing alive individuals in the treatment and the control experiment, respectively, corrected with the Abbott's folmula.

Tab. 3 Counts of positive biotest (with live insects) and mortality percentage in TREATED treatment for the different species and stages considered

Insect	Stage	Positive biotests (alive)	Mortality (%)
P. interpunctella	Eggs	0	100,0
P. interpunctella	Larvae	55	5,2
C. cautella	Eggs	0	100,0
C. cautella	Larvae	54	6,9
C. cephalonica	Eggs	0	100,0
T. confusum	Mix population	47	19,0
O. surinamensis	Mix population	19	67,2

Tab. 4 Counts of positive biotest (with live insects) and mortality percentage in UNTREATED treatment for the different species and stages considered

		TNT		LNT	
Insect	**Stage**	**Positive (alive) biotests**	**Mortality (%)**	**Positive (alive) biotests**	**Mortality (%)**
P. interpunctella	Eggs	2	0	2	0
P. interpunctella	Larvae	2	0	2	0
C. cautella	Eggs	2	0	2	0
C. cautella	Larvae	2	0	2	0
C. cephalonica	Eggs	2	0	2	0
T. confusum	Mix population	2	0	2	0
O. surinamensis	Mix population	2	0	2	0

*TNT=transfer untreated treatment - *LNT= Laboratory untreated treatment

Discussion

The test highlighted that an exposure to N_2 saturation at temperatures 15-18 °C for 21 days was not sufficient for a total control on mobile stages of all pests, while a total control of the *Lepidoptera* eggs, was observed. In fact no silk webs, feces or newborn larvae, even dead, were present in the biotests.

The population of *O. surinamensis* was the most susceptible to the treatment with 67.2% efficacy. The larvae of *P. interpunctella* were the least susceptible, 5.2% efficacy, similarly, the population of *C. cautella* with 6.7% efficacy. The treatment was barely effective on the population of *T. confusum*, with 19% efficacy.

References

NAVARRO, S., 2006. Modified atmospheres for the control of stored-product insects and mites. insect management for food storage and processing, 105-146.

CONYERS, S.T. AND C. H. BELL, 2004. Controlled atmospheres–A European perspective. In: Abstract of the International Conferance on Controlled Atmosphere and Fumigation in Stored Products, Gold Cost Australia.

AULICKY, R., KOLAR, V., PLACHY, J. AND V. STEJSKAL, 2016. Preliminary report on controlled nitrogen atmosphere in metal silo bin in the Czech Republic. Pp. 329–332. In: NAVARRO S, JAYAS DS, ALAGUSUNDARAM K, (Eds.) Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2016), CAF Permanent Committee Secretariat, Winnipeg, Canada

JIAN, Y., PENGCHENG, F., HAOJIE, L., XIAOPING, Y., YUE, L., JIANWU, D. AND S. QIANG, 2016. Application and development of controlled atmosphere with nitrogen in Chinese grain storage. Pp. 310–315. In: Navarro S, Jayas DS, Alagusundaram K, (Eds.) Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2016), CAF Permanent Committee Secretariat, Winnipeg, Canada.

FIELDS, P.G. AND N.D.G. WHITE, 2002. Alternatives to methyl bromide treatments for stored-product and quarantine insects. *ANNUAL REVIEW OF ENTOMOLOGY*, , **47.1**: 331-359.

FLEURAT-LESSARD, F., 1990. Effect of modified atmospheres on insects and mites infesting stored products. Food preservation by modified atmospheres, 21-38.

Effect of modified atmosphere on larval and pupal stages of *Rhyzopertha dominica* in stored chickpeas

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Abstract

The lesser grain borer, *Rhyzopertha dominica* (Fabricius), is a pest of stored chickpeas in Mexico. The control of this pest is based largely on the application of pesticides, but this strategy has important limitations: there are few active compounds available, there is a high risk of development of resistance to them and the residues left in the chickpeas have harmful effects on consumer health and on the environment. For this reason, an alternative strategy to pesticides for the conservation of stored chickpea was evaluated with the use of modified atmospheres (MA). The effect of three different MAs (50%, 70% and 90% CO_2, in air) on the larval and pupal stages of *R. dominica* were evaluated. To obtain larvae and pupae of *R. dominica*, eggs were incubated for a variable period of time until reaching the desired stage: 9-15 days for 1st and 2nd larval instar and 35-39 days for pupae. Tests were carried out by placing chickpeas containing a total of 15 larvae or pupae plus 50 g of healthy chickpea in small ventilated boxes. These ventilated boxes were individually placed inside of plastic bags (30 x 21 cm, Cryovac BB4L µm). Bags were filled with desired MA before sealing, which were previously prepared in a gas mixer (Witt Km 100-3M/MEM). A control treatment without MA was also included. To verify the CO_2 and O_2 content inside the plastic bags a gas analyzer (OXYBABY®) was used and the gas levels were determined at the beginning and at the end of the treatment. Plastic bags were opened at different periods of exposure (larvae up to 5 days; pupae up to 10 days) and ventilated boxes were kept until adult emergence to assess mortality. Results show that increasing the concentration and exposure time of CO_2 increases the mortality rate of larvae and pupae of *R. dominica* (Fig. 1). The most resistant developmental stage was the pupae, with an LD_{90} of 241 h (50% CO_2) compared to the larval stage with an LD_{90} of 22 h (90% CO_2). The tolerance of the MA is greater in the pupal stage due to the reduction of respiration in this stage.

Keywords: *R. dominica*, pest, chickpea, alternative strategy, modified atmosphere.

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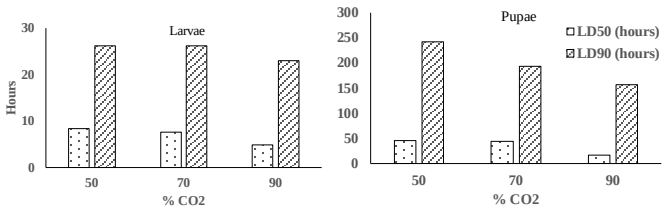

Fig 1. LD_{50} and LD_{90} of larval (left) and pupal (right) stage of *R. dominica* exposure at MA with 50, 70 and 90% of CO_2 on air.