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Hermetic storage as an alternative for controlling *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) and preserving the quality of cowpeas

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ABSTRACT

The aim of this study was to assess the use of modified atmosphere through hermetic storage in polyethylene silo bags and polyethylene terephthalate (PET) bottles as a technique to control *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae) and to preserve the quality of cowpeas during storage. Cowpea grains were stored in polyethylene silo bags, polyethylene terephthalate (PET) bottles and glass recipients (control) for 30, 60, 90 and 120 days. Each treatment was replicated four times. After each storage period, we assessed the insect infestation percentage and measured the moisture content, the bulk density, the germination percentage and the electrical conductivity of the grains. The percentage of infestation by *C. maculatus* for cowpeas stored in silo bags and PET bottles was low and did not exceed 4% during the entire storage period. In contrast, the percentage of infestation by *C. maculatus* increased in untreated cowpeas over the storage period. The moisture content, bulk density, germination percentage and electrical conductivity of the cowpeas were preserved in both hermetic storage systems that were tested for 120 days. To conclude, the hermetic storage of cowpeas using silo bags and PET bottles can efficiently control *C. maculatus* and preserve the quality of cowpeas for at least 120 days of storage.

1. Introduction

Cowpea, *Vigna unguiculata* (L.), is an edible herbaceous legume of great importance in several regions of the world, especially in the tropics and subtropics areas of the planet (Lopes et al., 2018). It is an important source of protein, carbohydrates, lipids, sodium, potassium and iron for the human diet (Cheng et al., 2013; Murdock et al., 2012). It is usually cultivated in the rainy season, but it is consumed throughout the year, thus creating the need for advances in storage technology (Ibro et al., 2014; Sanon et al., 2011; Baoua et al., 2012).

Callosobruchus maculatus (F.) (Coleoptera: Chrysomelidae: Bruchinae) is one of the most destructive stored-product insects of cowpea (Baoua et al., 2012; Lopes et al., 2018). It infests the seeds in the field and under storage (Souza et al., 2011; Cheng et al., 2013), causing considerable quantitative and qualitative losses (Vales et al., 2014). For its control, phosphine (PH_3), as well as pyrethroid and organophosphate insecticides, are mainly used (Gbaye et al., 2012; Lopes et al., 2016). However, the constant and, in several cases, improper use of these insecticides has favoured the development of resistance by several *C. maculatus* populations to commonly used insecticides (Odeyemi et al., 2006; Gbaye et al., 2016). Usually, small farmers sell cowpeas immediately after harvesting, when the market price is low, to avoid storage losses.

The storage in hermetically sealed polyethylene silo bags can be a promising alternative for the protection of stored cereal grains and legumes in commercial storage facilities, as well as on the farm (Jones et al., 2011; Freitas et al., 2016). The silo bag is a silo system made of flexible polyethylene plastic that is fully adaptable to any volume of grain. The bag is unrolled onto the ground, filled with grain using specialized equipment and sealed at both ends. They are used to increase storage capacity or create an alternative to

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traditional methods to reduce bottlenecks in the harvesting process (Ridley et al., 2011). Normally, the silo bags are 60 m long and 2.70 m in diameter. Approximately 200 tons of grains, such as corn and soybeans, can be stored in the silo bag for six to eight months (Abalone et al., 2011).

During storage in silo bags, the respiratory process of the biotic components of the ecosystem (grains, fungi and insects) consumes the oxygen (O_2), generating carbon dioxide (CO_2). An atmosphere rich in CO_2 and poor in O_2 can suppress the reproduction capacity and/or the development of insects and fungi, as well as the metabolic activity of grains, promoting the conservation (Moreno-Martinez et al., 2006; Navarro, 2012a; b; Cheng et al., 2013) and reducing the oxidation rate of the stored product (Santos et al., 2010; Carvalho et al., 2012). Hermetic storage in polyethylene terephthalate (PET) bottles is an alternative to silo bags for small-scale legume production. Storage in PET bottles enhances the preservation of the qualitative characteristics of cowpeas during storage. Being impermeable, PET bottles prevent gas exchanges with the environment (Freitas et al., 2011, 2016).

Although hermetic packages are widely used as a safe way of storage, there is a lack of information in the literature about the use of silo bags and alternative packages for the protection of cowpeas during storage in farms. Given the above, this study aimed to assess the efficacy of hermetic storage of cowpeas in polyethylene silo bags and polyethylene terephthalate (PET) bottles for the protection of cowpeas against *C. maculatus*. Moreover, the effect of hermetic storage on the quality characteristics of cowpeas during storage was investigated.

2. Material and methods

The experiment was conducted in the Federal Institute of Education, Science and Technology of Maranhão, in the city of Codó, state of Maranhão, Brazil. Grains of the BRS Guariba cultivar of cowpea with the following characteristics were used: insect infestation rate = 1.0%, moisture content = 12.1% wet basis (w.b.), bulk density = 844.3 kg m^{-3}, electrical conductivity = 110.3 μS cm^{-1} g^{-1} and 85% germination rate.

The cowpeas were packed in polyethylene silo bags and polyethylene terephthalate (PET) bottles. The bags had a capacity of 500 g and were made of the same 250-mm thick plastic used for the manufacturing of Silox TM (DuPont) silo bags. The bags were made of a three-layer plastic and were black on the inner side and white on the outer side with UV stabilizers. The bags were hermetically sealed with a multi-use sealing machine (hot bar 40/60 cm), and the bottles were properly sealed with a screw cap. The plastic layers of the bags are a mixture of high dense (HDPE) and low dense polyethylene (LDPE). The plastic bottles were reused transparent soda bottles with a capacity of 0.6 L and thickness of 270 mm. These were properly sealed with a screw cap and later sealed with beeswax, preventing gas exchanges. For the control treatment, the grains were placed in transparent glass containers with a capacity of 1.2 L and closed with an organza cloth, to allow gas exchange, as well as to prevent the exit or entry of insects into the containers.

Cowpeas were stored in silo bags, PET bottles and in control containers for 30, 60, 90 and 120 days, in climatic chambers at a temperature of 25 ± 2 °C with relative humidity of 70 ± 5%. Every 30 days, four packages of each treatment were opened to analyse the infestation by insect pests, the moisture content, the bulk density, the germination percentage and the electrical conductivity.

To assess the insect infestation rate by insect pests, three samples of 100 cowpea beans were retracted from each package, and four packages of each treatment were examined (n = 12). These samples were immersed in water for 24 h, enough time to soften the grains. After this period, the grains were removed from the water, dried on filter paper, cut and individually examined. Grains considered infested were those containing young or adult insects and/or exit orifices of insect pests, according to recommendations of the Rules for Seed Analysis (BRASIL, 2009).

Moisture content of the grains was determined using the oven method according to the norms of the ASAE (2004), which suggest the use of a forced air oven at 130 ± 1 °C for 72 h for three samples of 30 g of cowpeas removed from each package (out of four packages examined for each treatment (n = 12)). Seventy-two hours was used for samples of 30 g of grains. The weighing was made on a scale with a resolution of 0.01 g, and the analyses were conducted in triplicates and the results expressed as wet basis.

The bulk density was determined with the aid of a hectolitre scale, model "Determinador de PH" (DPH), manufactured by Dalle Molle, (Balanças Dalle Molle Ltda), with a load capacity of a quarter of litre (250 mL). The analyses were conducted according to the methodology described by the Rules for Seed Analysis (BRASIL, 2009), performed in triplicates and the results expressed in kg m^{-3}. The determinations were made through the analysis of three samples taken from the four packages of each treatment (n = 12).

The germination test was conducted according to the Rules for Seed Analysis (BRASIL, 2009) using four samples 50 cowpea beans for the four packages of each treatment (n = 16). The substrate used was "germitest" germination paper, moistened with distilled water at a ratio of 2.5 times the weight of the paper. The cowpea grains were laid out over two sheets of the germination paper, subsequently covered by another sheet of the same paper and packed in forming rolls. The rolls were placed in a vertical position inside a germinator and kept at a temperature of 25 ± 1 °C. The final count was made after nine days, considering the normal seedlings, and the data were expressed as the mean percent of germination.

The electrical conductivity of the solution containing the beans was measured using the Cup System or Mass Conductivity (Vieira et al., 2002). To measure the electrical conductivity of the solution containing the grains, the tests were conducted with three samples of 50 grains from the four packages of each treatment (n = 12). The grains were weighed on an analytical scale with a precision of 0.01 g and placed in plastic cups of 200 mL, to which 75 mL of deionized water was added. The cups were placed in a B.O.D incubator at a temperature of 25 °C for a period of 24 h. Immediately after this period, the cups were removed from the chamber for measurement.

The experiment was conducted using a completely randomized design, with subdivided blocks of four replicates. The blocks represented the storage systems (silo bags, PET bottles and control containers), and the sub-blocks were the periods of storage (initial characterization, and at 30, 60, 90 and 120 days). The data were submitted to analyses of covariance ($P < 0.05$). Since the interaction between storage conditions and periods of storage was significant, we conducted further analyses on the data. The values obtained were submitted to a regression analysis as a function of time.

The regression models were chosen based on the significance of the regression coefficients, using the *t*-test on the coefficient of determination (R^2), and based on biological phenomena. Regardless of the significance of the highest degree of interaction, we decided to conduct further analyses of the interaction due to the biological interest of the study. We estimated the Pearson correlation coefficient ($P < 0.05$) between the rate of infestation by insects and the moisture content, bulk density, germination percentage and electrical conductivity of the grains of cowpea.

3. Results

We detected significant variations between the storage systems (silo bags, PET bottles and control containers) regarding the rate of