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The effect of small leaks, grain bulk, and the patching of leaks on the performance of hermetic storage

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ABSTRACT

Hermetic storage containers are often used by farmers to protect their harvested grain from insect damage and ultimately stop insect population development. Sometimes holes in a storage container are created by insects or by accident; such holes may reduce the effectiveness of the hermetic storage unit. Using cowpea grain and the cowpea bruchid, *Callosobruchus maculatus* (F), we investigated the degree to which holes in a hermetic storage container wall affect the level of grain damage. When there were low numbers of holes, seed damage increased markedly with each additional hole. The grain itself contributed a barrier to oxygen diffusion through the grain mass. If holes in the container wall were patched with a single layer of HDPE film, grain damage was indistinguishable from that seen under full hermetic conditions. We provide evidence that a single layer of woven polypropylene contributes a small but measurable barrier to oxygen penetration into the container.

1. Introduction

The Purdue Improved Crop Storage (PICS) system was developed to provide affordable hermetic containers for weevil-safe storage of cowpea grain, *Vigna unguiculata* (Walpers), against the bruchid, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae) in the Sahel region of West Africa (Murdock et al., 2003, 2012; Sanon et al., 2011). Each PICS bag is comprised of three separate bags, two high density polyethylene (HDPE) liners nested inside a woven polypropylene bag (Baoua et al., 2012; Murdock et al., 2012; Baributsa et al., 2013). The two inner bags are 80 μm thick. The HDPE layers, while not perfectly impermeable, greatly inhibit O_2 and CO_2 exchange between the air spaces within the bags and the atmosphere (Kjeldsen, 1993). Metabolism of insects already present in the grain when it is put into the bag leads to much lower internal levels of O_2 and higher levels of CO_2. With reduced available O_2 (hypoxia) and elevated CO_2 (hypercarbia), the insects in the grain cease feeding, growing and developing and often die (Murdock et al., 2012). Population growth is thereby arrested. If the technology is applied early in the storage season, it results in minimal or no damage to the grain (Murdock et al., 2012; Cheng et al., 2012; Baoua et al., 2013a).

PICS HDPE liners sometimes acquire small holes during storage, most frequently in the inner liner (Baoua et al., 2012). Cases in which both HDPE layers have been penetrated by insects have also been observed but are much less frequent. Holes in the HDPE film often result when an infested seed with a pupal cell happens to be pressed against the inner HDPE membrane under the pressure exerted by the bulk grain in the bag. When the adult leaves its pupal cell it cuts its emergence hole through the seed testa and continues on through the plastic membrane, making a round emergence hole in that as well as in the testa (Baoua et al., 2012).

Holes in the HDPE liners of PICS bags may allow insect development to occur in the grain adjacent to the hole due to the influx of O_2 diffusing through the opening. This can ultimately lead to an increased level of damage in the stored grain. However, if the second, outer layer of HDPE were still intact, i.e., if only the inner layer has been penetrated by an emerging insect, this intact layer may sufficiently retard O_2 influx to prevent larval development and damage near the hole. If there are multiple holes in an HDPE liner instead of just one, one would expect increased numbers of insects to develop. In that case, one would expect a positive relationship between the number of holes in the HDPE film and the number of insects that develop.

Insects often aggregate at the top of grain stores (Navarro et al., 1984; Driscoll et al., 2000) and unpublished field observations of the PICS technology sometimes noted large numbers of insects gathered at the top of PICS bags (Baoua, personal communication).

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This increased insect density may be a result of higher oxygen availability at the top of the bags or may merely reflect an escape behavior of the insects. Oxygen availability may be at its lowest toward the center of the grain mass due to (1) slow diffusion rates through the grain (Singh et al., 1984; Shunmugam et al., 2005; Huang et al., 2013; Haugh and Isaacs, 1967) and (2) oxygen use by insects closer to the outer perimeter. Packing grain tightly, decreasing temperature, and increasing moisture content will further reduce oxygen availability within the center of the grain mass (Singh et al., 1984; Driscoll et al., 2000; Shunmugam et al., 2005).

Differences in O_2 concentration at points within PICS bags may occur and influence insect metabolism and development. Such differences may depend on the location of infested seeds within the container. If more oxygen were available in a particular region of the grain mass (likely toward the surface of the grain mass or near a hole) more damage might occur. In an earlier study, insect populations near an air inlet developed at a faster rate than those further from the inlet (Driscoll et al., 2000). Less damage is likely farther from the surface of a bulk or more distant from holes and imperfections in the container walls.

The grain itself may act as a barrier to the diffusion of oxygen into the center of a mass of stored grain. If that case, we would expect there to be a gradient of decreasing seed damage in relationship to the distance from the source of oxygen. The present study sought to (1) determine the degree to which holes in hermetic containers compromise protection against storage insects; (2) determine the extent to which cowpea grain acts as a barrier to gas diffusion and thus contributes to suppressing the growth of cowpea bruchid populations, and; (3) determine the effectiveness on patching small holes with HDPE or woven polypropylene in terms of hermetically protecting the grain from insect damage in a container. Point (3) was framed as the hypothesis that the double HDPE layer is one key to the performance of PICS bags and that the woven outer polypropylene layer makes a small but real contribution to the barrier properties of the PICS bag.

2. Materials and methods

All trials were conducted at Purdue University (West Lafayette, IN, USA) and were conducted in three parts over 70-day storage periods. This 70-day time frame was selected to permit two whole generations of cowpea bruchid to develop. Trial one, carried out from 26 September 2012 to 5 December 2012, examined the effect that the number of small bruchid-size holes ($r = 0.508$ mm) in a container's walls had on the performance of hermetic storage to protect cowpea grain against the cowpea bruchid. Trial two, carried out from 11 January 2013 to 22 March 2013, examined the effect of grain bulk thickness on the performance of hermetic storage. Trial three, carried out from 17 January 2014 to 28 March 2014, investigated whether covering a single, small hole ($r = 0.508$ mm) with either an 80um thick patch of HDPE or with woven polypropylene was sufficient to protect cowpea grain from the cowpea bruchid.

2.1. The effect of small holes on hermetic storage performance

Cowpea bruchids were obtained from a laboratory colony maintained on cowpea grain that had been started with insects from Niger, West Africa. California black-eyed cowpea grain, variety #8046 (Wax Co., MS, USA) was used in all trials. Prior to use the grain was held in a freezer at 0 °C for one week to kill any insects living in it. One week before setting up each experiment, 10 kg of cowpea grain was removed from the freezer and distributed in two, 17 L buckets. One bucket was heavily infested with *C. maculatus* adults from the laboratory colony, and the second, with no insects present, was hermetically sealed and returned to the freezer. Nine days later it was removed to allow the temperature to equilibrate. On the tenth day, the adult bruchids in the infested grain were removed via sifting. The two quantities of grain were mixed together on a large tarp to create a 10 kg, 50:50 mixture of infested and uninfested cowpea. This mixture was then sampled (4 samples of 100 seeds each) to determine the initial infestation level. The average initial infestation level for the first trial was 27.85% ± 0.64 of the seeds carrying at least one egg.

PVC pipes with an inner diameter of 3.81 cm were cut into forty 10 cm sections using a chop saw. A wireless power drill was used to bore small holes in a regular pattern in each pipe. The size of the drill bit used (#60, $r = 0.508$ mm) left a hole that was only slightly smaller than the emergence hole in the testa of a cowpea seed made by an emerging *C. maculatus*. This size hole prevented the escape of adult insects while still closely imitating the size of an emergence hole. Ten sets of four pipe sections were used, each set having a different number of holes. The treatments were 0, 1, 2, 4, 6, 8, 12, 24, 36, and 48 holes per pipe section. The holes for each treatment were drilled evenly spaced from the ends of the pipe and around the diameter of the pipe (e.g., for the pipe with two holes the holes were drilled on opposite sides of the pipe from each other and one was spaced 2.6 cm left of center and the other 2.5 cm right of center).

The pipe sections were filled with the infested cowpea mixture, then capped with tight-fitting PVC caps coated with high vacuum grease (DOW CORNING®, Midland, MI, USA). The replicate pipes was stored in a complete, random block design and left undisturbed in an environmental chamber on a cart divided into shelves. The chamber conditions were 25 °C, 40% relative humidity (RH), and with a 12:12 light/day (LD) cycle.

After 70 days of storage the tube sections were transferred to a freezer and held at 0 °C for one month to kill the surviving insects. Results were evaluated by emptying each pipe into an opaque cup from which a 100 seed sample was selected without looking inside the cup to limit visual selection bias. Grain damage was assessed using three different measures: weight of the sample, number of grains containing at least one emergence hole, and total number of holes in the 100 grain sample. All parameters were evaluated for significance using a one-way ANOVA and the Tukey–Kramer HSD test of comparative means in JMP 10 statistical software (SAS, Cary, NC, USA).

2.2. The effect of distance from an air source on insect damage

The second trial investigated the effect that distance from an air source within a grain bulk has on insect population distribution and development. We created a 100 kg mixture of infested and uninfested cowpea grain, as described above. The initial infestation was determined by counting the number of grains carrying at least one egg. Approximately 27.23% ± 0.51 of the grain in the resulting grain mixture was infested.

The following treatments were applied to PVC tubes measuring 1.5 m in length and having an inner diameter of 3.81 cm: (1) both ends were hermetically sealed with PVC caps coated with high vacuum grease (DOW CORNING®, Midland, MI, USA). This treatment acted as a hermetic storage control in which there was no access to ambient air; (2) one end was hermetically sealed with a PVC cap coated with vacuum grease and one end was covered only with a layer of cheesecloth held in place by a rubber band and hose clamp; (3) both ends were covered with cheesecloth; (4) both ends were hermetically sealed with PVC caps coated with vacuum grease with a single hole drilled in the center of one cap using a power drill, drill bit size #60 ($r = 0.508$ mm). This last treatment examined the effect of one emergence hole-sized leak on the effectiveness of hermetic storage; (5) both ends were covered with cheesecloth