



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.

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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 μ m thick. The HDPE layers, while not perfectly impermeable, greatly inhibit O₂ and CO₂ 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 O₂ and higher levels of CO₂. With reduced available O₂ (hypoxia) and elevated CO₂ (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 O₂ 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 O₂ 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 O₂ 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 80 μ m 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 $27.85\% \pm 0.64$ 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 $27.23\% \pm 0.51$ 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

with small holes ($r = 0.508$) drilled every 25.4 mm around and down the length of the tube. This last treatment acted as a control providing extensive access to ambient air.

The twenty 1.5 m long PVC tubes were filled with the infested grain mixture. Each tube was tapped against a hard surface while being filled to eliminate airspaces and make sure the grain was adequately compacted. The tubes were then held in an environmental containment chamber, as described above, with each block kept on a separate shelf. Five treatments were used with four replicates each and arranged in a complete, random block design.

After 70 days of storage, the tubes were transferred to a freezer at 0 °C for 1 month. Each 1.5 m tube was then cut into 7.5 cm sections using a chop saw. After each cut was made pieces of cardboard were placed between the blade and the openings prevent grain spillage while removing the newly cut section. The grain in each cross section was subsequently assessed for damage relative to the distance from the end of the pipe. A total of 100 seeds were selected blindly and at random. Damage was determined by counting the number of emergence holes per 100 seed sample. The samples at each distance along the pipe were evaluated for significance using one-way ANOVA and the Tukey–Kramer HSD test of comparative means in JMP 10 statistical software (SAS, Cary, NC, USA).

2.3. The effect of patching small holes on insect damage

The third trial investigated the effectiveness of small, 2 × 2 cm HDPE or woven polypropylene patches in blocking bruchid growth and development by acting as oxygen barriers. We used the previously described procedure to create a 10 kg mixture of infested and uninfested cowpea grain. The initial seed infestation mixture had 25.21% ± 0.81 of the grains containing one or more eggs.

Twelve PVC pipes each with an inner diameter of 3.81 cm and length of 10.16 cm were cut with a chop saw. A single, small hole ($r = 0.508$ mm) was drilled halfway along the length of each pipe. The treatments were (1) four pipes with a 2 × 2 cm square of 80µm thick HDPE covering the small hole, (2) four pipes with a 2 × 2 cm square of woven polypropylene material covering the hole, and (3) four pipes with no covering over the hole.

The twelve pipes were filled with the infested cowpea mixture and were sealed with tightly fitting PVC caps coated with high vacuum grease. The pipes were stored in the environmental chamber described above for 70 days. At the end of the storage period, samples were heated at 65 °C for 24 h to kill the insects present. As in the previous trials, damage was determined by counting the number of emergence holes per 100 seed sample. The samples were analyzed using one-way ANOVA and the Tukey–Kramer HSD test of comparative means in JMP 10 statistical software (SAS, Cary, NC, USA).

3. Results

3.1. The effect of small holes on hermetic storage performance

There was a significant ($P < 0.0001$, $F = 200.906$, $df = 9$), positive correlation between the number of holes in the pipe and the level of grain damage (Table 1). As the number of holes in the pipe increased, the number of emergence holes also increased. Grain damage reached a plateau when 24 or more holes were present in the pipe.

A single, bruchid-sized hole in the PVC container wall caused a statistically significant increase in damage over the control having no holes ($P < 0.0001$, $df = 3$). During the 70 days of storage, the number of emerged adults per 100 seed sample increased from 3.5 insects reaching adulthood in the hermetically sealed control pipes to 159.0 ± 7.08 adults emerging per 100 seed sample in the pipes with only one hole.

Table 1

The effect of small holes ($r = 0.508$ mm) in the walls of a storage containers on *C. maculatus* population development in cowpea grain.

| Holes per storage container (n = 4) | Number of emergence holes per 100 seed sample | Percentage of damaged seeds per sample (%) | Weight of 100 seed sample (g) |
|-------------------------------------|---|--|-------------------------------|
| 0 | 3.5 ± 3.18a | 3.0 ± 2.68a | 21.90 ± 0.32a |
| 1 | 159.0 ± 7.08b | 79.25 ± 1.11b | 20.36 ± 0.24 ab |
| 2 | 291.0 ± 18.53c | 95.5 ± 1.50c | 18.26 ± 0.73abc |
| 4 | 437.0 ± 13.08d | 96.25 ± 1.03c | 16.76 ± 0.92bcd |
| 6 | 481.0 ± 26.88de | 97.75 ± 1.26c | 14.65 ± 1.41cde |
| 8 | 548.75 ± 33.33e | 97.0 ± 1.08c | 12.52 ± 0.99def |
| 12 | 668.5 ± 26.08f | 99.75 ± 0.25c | 11.11 ± 0.71ef |
| 24 | 843.0 ± 28.74g | 99.0 ± 0.58c | 10.66 ± 1.63ef |
| 36 | 862.5 ± 22.12g | 100.0 ± 0.00c | 8.60 ± 0.59f |
| 48 | 899.0 ± 16.61g | 99.75 ± 0.25c | 8.93 ± 0.16f |
| Anova (F, P) | (F = 200.906, P < 0.0001) | (F = 657.7081, P < 0.0001) | (F = 28.3395, P < 0.0001) |

Means followed by the same letter within a column are not significantly different from each other at a 5% probability level by the Tukey–Kramer HSD test. Error terms represent ± one standard error mean.

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

In the second set of trials, we investigated the effect distance from a source of leakage has on grain damage (Table 2). This experiment was analyzed using the total number of emergence holes per sample, the measure that had proven to be the most accurate indicator of damage in the previous experiment. Damage to grain in the hermetically sealed pipes was extremely low and independent of position in the tube ($p = 0.6263$, $F = 0.6265$, $df = 19$). Damage was independent of distance from the large inlet in the heavily perforated pipe ($P = 0.9997$, $F = 0.2232$, $df = 19$). We believe that the hermetically sealed pipe mimics conditions in hermetic containers when the oxygen supply is highly limited, whereas the perforated pipe mimics conditions in hermetic containers in which there are one or more localized micro-leaks. Distance from a fresh air supply had a significant effect (ANOVA: $P < 0.0001$, $F = 36.2625$) on damage level in the remaining treatments of one end open, both ends open, and a single small hole in one end.

Analysis showed that the pipe with a single open end had more damage between 0 and 15 cm from the opening than in the remaining 135 cm of stored grain column (Table 2). Grain damage beyond 30 cm from the open end of the column was nearly undetectable and comparable to the hermetic control. The double open-ended treatment had observable levels of damaged seeds between 0 and 30 cm and between 112.5 and 142.5 cm, with relatively undamaged grain in the center section of the pipe. This double, open-ended treatment had more damaged seeds between 0 and 7.5 cm than was seen in the pipe with a single open end.

The treatment pipes with a single, 0.508 mm radius hole in the cap had fewer damaged grains close to the opening in comparison to the pipes with completely open ends. Relative to the rest of the pipe, damage was slightly elevated in the first 7.5 cm of stored grain, but little additional damage was seen deeper in the column of grain, where damage was comparable to levels seen in the hermetically sealed pipe. Likewise, there was less damage in the first 7.5 cm of the single-hole treatment pipes compared to either treatment with open ends.

3.3. The effect of patching small holes on hermetic storage performance

Patching (Fig. 1) had a significant effect on the level of grain damage (ANOVA: $F = 149.72$, $d.f. = 3$, $P < 0.0001$). The pipes with

Table 2The effect of distance from an air source on *C. maculatus* population development in cowpea grain.

| Distance from end of pipe (cm) | Number of emergence holes per 100 seed sample (n = 4) | | | | |
|--------------------------------|---|------------------------------|-----------------------------|-----------------------------|-----------------------------|
| | Single open end | Double open ends | Sealed ends | Perforated | Single hole in sealed end |
| 0 | 277.5 ± 30.70a | 453.0 ± 50.11a | 0.3 ± 0.25a | 459.5 ± 28.58a | 18.5 ± 7.58a |
| 7.5 | 174.0 ± 3.46b | 233.0 ± 24.18b | 0.0 ± 0.00a | 451.0 ± 44.20a | 14.0 ± 4.20 ab |
| 15 | 104.25 ± 12.76c | 83.5 ± 18.07c | 0.3 ± 0.25a | 428.0 ± 62.69a | 8.0 ± 2.52abc |
| 22.5 | 42.25 ± 11.23d | 45.0 ± 4.22c | 0.0 ± 0.00a | 433.0 ± 56.60a | 9.5 ± 2.10abc |
| 30 | 30.5 ± 7.96d | 14.5 ± 1.04c | 0.0 ± 0.00a | 406.5 ± 67.24a | 7.0 ± 1.87abc |
| 37.5 | 23.5 ± 4.97d | 13.7 ± 3.09c | 0.0 ± 0.00a | 386.3 ± 47.77a | 7.0 ± 1.08abc |
| 45 | 15.25 ± 2.72d | 16.5 ± 1.04c | 0.0 ± 0.00a | 405.8 ± 46.78a | 3.5 ± 0.65bc |
| 52.5 | 12.75 ± 2.21d | 16.0 ± 2.65c | 0.0 ± 0.00a | 410.8 ± 48.55a | 3.5 ± 1.44bc |
| 60 | 9.25 ± 3.35d | 10.5 ± 2.33c | 0.0 ± 0.00a | 407.0 ± 68.17a | 2.8 ± 0.48bc |
| 67.5 | 8.00 ± 2.04d | 13.0 ± 3.58c | 0.5 ± 0.50a | 423.5 ± 33.04a | 3.8 ± 0.75bc |
| 75 | 5.25 ± 1.18d | 16.0 ± 4.08c | 0.0 ± 0.00a | 428.3 ± 50.76a | 3.0 ± 0.91bc |
| 82.5 | 3.00 ± 1.08d | 9.3 ± 2.95c | 0.0 ± 0.00a | 427.3 ± 57.52a | 4.5 ± 1.32bc |
| 90 | 3.75 ± 1.31d | 12.5 ± 5.20c | 0.0 ± 0.00a | 388.0 ± 48.77a | 1.5 ± 0.50c |
| 97.5 | 3.25 ± 2.59d | 14.75 ± 4.42c | 0.0 ± 0.00a | 420.3 ± 57.00a | 1.0 ± 0.58c |
| 105 | 2.25 ± 1.60d | 15.5 ± 8.92c | 0.0 ± 0.00a | 435.8 ± 60.94a | 2.8 ± 0.25bc |
| 112.5 | 1.25 ± 1.25d | 24.3 ± 12.17c | 0.0 ± 0.00a | 425.5 ± 61.24a | 1.3 ± 0.95c |
| 120 | 2.25 ± 2.25d | 44.0 ± 17.33c | 0.0 ± 0.00a | 414.5 ± 41.36a | 2.5 ± 1.19bc |
| 127.5 | 3.50 ± 2.02d | 121.3 ± 29.27bc | 0.5 ± 0.50a | 491.8 ± 72.51a | 1.3 ± 0.63c |
| 135 | 0.25 ± 0.25d | 217.5 ± 29.18b | 0.0 ± 0.00a | 431.5 ± 40.18a | 2.0 ± 1.68c |
| 142.5 | 0.75 ± 0.25d | 393.8 ± 62.53a | 0.0 ± 0.00a | 412.8 ± 24.36a | 1.8 ± 0.75c |
| Anova (F, P) | (F = 72.9715, P < 0.0001) | (F = 36.2625, P < 0.0001) | (F = 0.6265, P = 0.6263) | (F = 0.2232, P = 0.9997) | (F = 4.1143, P < 0.0001) |
| df = 19 | | | | | |

Means followed by the same letter within a column are not significantly different from each other at a 5% probability level by the Tukey–Kramer HSD test. Error represents ± one standard error of the mean.

the HDPE patch contained no damaged seeds, and was significantly protected compared to grain in the pipes with no patch ($P < 0.0001$) and the woven polypropylene patch ($P < 0.0001$). The pipes with the woven polypropylene patch had reduced seed damage compared to the pipes with no patch ($P < 0.0030$), but significantly more damage than the pipes with the HDPE patch ($P < 0.0001$).

4. Discussion

4.1. The effect of small holes on hermetic storage performance

Increasing the number of holes in the walls of a hermetic container resulted in an increase in seed damage. When the

number of holes was very small, each additional hole led to large increase in damage. For example, a large increase in the number of bruchid holes per 100 grains was observed between the control pipes, which had no openings (3.5 holes per 100 grains) and pipes with a single opening for air (159 holes per 100 grains). The level of damage increased with greater numbers of holes up to 24 holes per pipe. There was no substantial increase in damage above this level. At 24 holes, there may have been enough oxygen available to meet the needs of the developing insects. Any additional leakage above that level may have been in excess of the insects' needs and other resources may have become limiting. One of these may have been the carrying capacity of the seeds. At 8 to 10 holes per grain, there may not have been enough available food to support additional developing larvae, thereby limiting the attained population size.

This trial shed light on the importance of selecting an appropriate measurement method for interpreting the effect of a treatment. Mean grain weight and the total number of emergence holes per 100 grains proved to be superior indicators of the treatment effect than the percentage of damaged grains. The percentage of damaged grains was a less useful measure because damage levels were not significantly different between treatments. Of the three measurements, the total number of emergence holes per sample was the most reliable. The total number of emergence holes per grain is also a good measure because each hole results from one adult insect; this value allows for the adult population in the container to be estimated.

It may be useful to point out that there is a source of error built into the weight measure: Grain samples with very high numbers of emergence holes not only experienced heavy damage but also had observable mold growth. The damaged and moldy grain with its increased moisture content weighed more than damaged grain without mold. Mold presumably resulted from the increased heat and humidity associated with heavy insect infestations (Denmead and Bailey, 1966; Driscoll et al., 2000).

The increase in damage when there was only a single hole in the container wall suggests that even one hole in a hermetic storage container is sufficient to cause an increase in localized damage. This

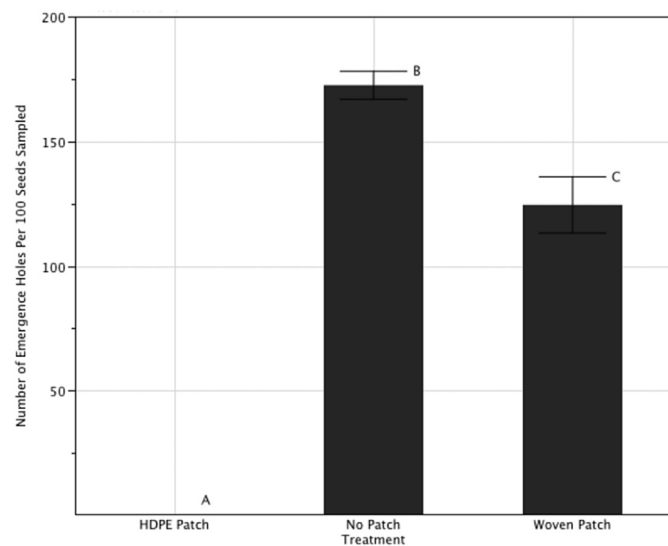


Fig. 1. The effect of patching small holes ($r = 0.508$ mm) in a storage container on *C. maculatus* emergence in cowpea grain after three months of storage. Error bars represent ± one standard error.

increase in damage is large enough to warrant patching or immediate transfer of store contents to a new container. Real world examples support this finding. A study conducted in Niger suggests that farmers using hermetic storage bags have discovered that taping over holes maintains bag storage performance (Baributsa et al., 2014).

We point out that the small volume of the PVC containers ($v = 116 \text{ cm}^3$) we used in these experiments may bias this trial in favor of greater insect damage because of the favorable surface-to-volume in the small-sized containers. The effect of air leakage on insect population growth and grain damage would probably be less in larger containers.

4.2. The effect of distance from air source on insect damage

The degree of grain damage observed in the grain bulk thickness trials depended on both the number of holes permitting airflow and the distance the grain was from the hole. The greater level of damage observed in the double open-ended pipes compared to pipes with only a single-end opening is likely due to increased ventilation that occurs when both ends are open. Additional O_2 availability in the pipe allowed for greater larval development and resulted in an increase in damage when both ends were open. In the pipes with one or both ends open, there was a negative relationship between seed damage and distance from the opening.

These observations support our hypothesis that damage in a grain store is more likely to occur at the surface, where access to contiguous air is greater. Our results are consistent with other authors' observations of increased population density toward the top of a grain mass (Driscoll et al., 2000).

We observed that a single, bruchid-sized hole provides sufficient oxygen to allow localized insect development and grain damage. As the distance from the holes increased damage nearly disappeared. This indicates that small holes do allow for limited insect development, but only in grain close to the hole.

In the first set of experiments investigating the effect of small leaks on storage performance, the treatment with one hole produced 159.0 ± 7.1 holes per 100 seed sample. This damage was observed in experiments using a low volume ($v = 116 \text{ cm}^3$) storage container in which the maximum distance from the hole was only 3.8 cm. This short distance may explain the high level of damage in comparison to the one-hole treatment in the second set of experiments. In the short PVC pipes, oxygen had to follow a relatively short diffusion path to reach developing insects. In sum, one bruchid sized hole appears to have a large, but only localized effect on grain damage. Beyond about 7.5 cm from the hole the grain experiences no more damage than is seen under nearly ideal hermetic conditions.

Our results indicate that increasing distance from a hole in a bag wall is associated with reduced insect damage. The diffusion path within the grain bulk together with insects developing (and respiring) near the hole are sufficient to reduce penetration of oxygen deeper into the grain bulk and thus from reaching insects a greater distance away. If a hole is created in a commercial hermetic storage container, grain near the hole will become damaged, but the damage will be localized to the space near the hole. Grain further away will remain intact and useful for consumption or sale.

4.3. The effect of patching small holes on hermetic storage performance

The results of the third trial showed that it is possible to patch damaged hermetic storage containers and restore their

effectiveness. Patching a small hole in a hermetic container with an 80 μm HDPE plastic film patch is sufficient for preserving the hermetic integrity of the container. Damage to the stored grain is thereby prevented. Further, our results show a woven polypropylene layer similar to the material used in conventional grain storage bags does indeed contribute a barrier to gas diffusion, and thus helps decrease damage when a hole is present in both the HDPE layers. These results demonstrate that a woven bag can be used to encapsulate a hermetic storage system to provide physical protection from puncture and provide additional grain storage protection against insects if a hole were to develop.

5. Conclusions

Hermetic storage of cowpea grain infested with *C. maculatus* stored under hermetic conditions prevents population expansion and arrests damage at essentially the level already present when the grain is put into storage. An increase in the number of point leaks in the walls of hermetic containers leads to an increase in insect damage but only in the vicinity of the leaks. When there is substantial access to O_2 , by contrast, the food supply rather than the oxygen supply probably limits the number of insects and the damage level. As the distance from the hole in the container wall increases the damage to grain decreases. This relationship indicates that larger containers are better than smaller ones for storage, as would be expected from the more favorable surface-to-volume relationship of larger containers compared to smaller ones of the same shape. The grain itself hinders oxygen diffusion. If a hole occurs in a container wall, the damage is localized because the grain surrounding the hole contributes a secondary barrier and helps slow penetration of O_2 . Small holes that appear in hermetic containers can be easily patched with HDPE or tape to reseal the container. The value of a double HDPE layer resides in the fact that both layers have to be penetrated even for local damage by insects to occur. A tertiary protective layer around a hermetic container such as a woven polypropylene bag contributes in a small but significant way to the hermetic properties of a container.

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