Storage of pigeonpea grain (*Cajanus cajan* (L.) Millsp.) in hermetic triple-layer bags prevents losses caused by *Callosobruchus maculatus* (F.) (*Coleoptera*: *Bruchidae*)

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Abstract

Dry pigeonpea grain is susceptible to infestation from the cowpea bruchid during storage. This study investigated the potential of Purdue Improved Cowpea Storage (PICS™) and polypropylene (PP) bags for reducing insect infestation under ambient laboratory conditions for six months. Some of the bags were artificially infested with cowpea bruchid *Callosobruchus maculatus* (PICS1, PP1) while others were not (PICS0, PP0). In an additional trial, PP bags containing pigeonpeas were treated with Actellic Super® dust before being artificially infested (PP1Ac). Moisture content, number of live *C. maculatus* adults, grain damage, and weight loss were determined at monthly intervals. Temperature and relative humidity (RH) in the PICS and PP bags were monitored continuously, while the oxygen and carbon dioxide concentrations in the PICS bags were measured at regular intervals. After six months moisture contents of pigeonpea grain stored in PP or PICS bags were not significantly different, and remained below 13%, the maximum required for safe storage of pigeonpeas. Adult *C. maculatus* populations increased rapidly in the PP bags, causing heavy seed damage. Percent damage after six months was 55, 96 and 76%, in PP0, PP1 and PP1Ac, corresponding to weight losses of 13.0, 26.2 and 13.5%, respectively. Actellic Super dust did not fully protect pigeonpeas against *C. maculatus*. By contrast, PICS bags halted multiplication of *C. maculatus* and subsequent seed damage and weight loss. PICS bags are an effective tool for preserving pigeonpeas against *C. maculatus* attack and their performance is superior to that of a single treatment with Actellic Super® dust.

Keywords: urdue Improved Cowpea Storage (PICS™) bags, polypropylene bags, Actellic Super® dust

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.), also known as red gram, is an important food legume crop in semi-arid tropical and subtropical farming systems (Shanower et al., 1999). The crop is harvested only once each year, which means that storage is an important component of the pigeonpea postharvest system. Bruchids of the genus *Callosobruchus* (*Coleoptera: Bruchidae*) including *C. chinensis* (L.), *C. maculatus* (F.) and *C. analis* (F.) are major causes of losses in stored pigeonpeas (Singh and Jambunathan, 1990). Various physical and chemical pest management methods are employed by farmers to preserve their grain (Obeng-Ofori, 2011). These strategies, however, are often not successful for long-term storage and are generally only suited for small quantities of grain. Use of chemicals as residual insecticides or fumigants is also common (Obeng-Ofori, 2011). At the off-farm level, the chemical control protocols, nonetheless, raise economic, technical, and safety concerns (Casida and Quistad, 1998).

Hermetic storage for grain preservation has been used since ancient times (De Lima, 1990) and may be an effective means for controlling insect infestations on stored produce (Navarro et al., 1994). The Purdue Improved Cowpea Storage (PICS™) bag has been shown to perform well in the control of *C. maculatus* in infested cowpeas. The PICS bag, which is a triple-layer bag consisting of an ordinary woven polypropylene (PP) bag lined with two

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layers of relatively low permeability polyethylene (HDPE) which can be tied shut, ensures effectively airtight conditions are created, and a modified atmosphere is generated through the respiratory action of the seeds and enclosed life-forms, particularly bruchids (Murdock et al., 2003). The present study was conducted to assess the performance of PICS™ bags as an alternative chemical-free storage technology for pigeonpeas. The performance of PICS bags was compared with that of PP bags under scenarios simulating storage re-infestation or cross-infestation as is commonly witnessed in many rural on-farm storage structures in Africa.

MATERIALS AND METHODS

Materials

Storage experiments were conducted at the International Centre of Insect Physiology and Ecology (ICIPE) Duduville campus in Nairobi in Kenya. Pigeonpea grain, cultivar 'Kat 777', was purchased at the Nyamakima market in Nairobi. The grain was fumigated with aluminium phosphide tablets containing 33% w/w phosphine (Shenzhen Carson Agrochemical Co. Ltd, Shenzhen, China). PICS bags of 50 kg capacity were supplied by Lela Agro Industries Limited (Kano, Nigeria) whereas PP bags were purchased from a local grain trader in Nyamakima market in Nairobi.

Bagging, storage and sampling

Both PICS and PP bags were filled with 20 kg of fumigated pigeonpeas and grouped into two and three sets, respectively. In one set of PICS bags and one set of PP bags, 100 un-sexed *C. maculatus* adults taken from a laboratory culture were introduced. These treatments were labelled PICS1 and PP1, respectively. In the second set of PICS bags and second set of PP bags no insects were introduced. These were labelled PICS0 and PP0. In the third set of PP bags the pigeonpeas were admixed with Actellic Super® (Pirimiphos methyl (1.6 g 100 g^{-1}) + permethrin (0.3 g 100 g^{-1}) at rate of 0.55 g 1000 g^{-1} of pigeonpeas, and 100 adults of *C. maculatus* were introduced. This treatment simulated insecticide preserved pigeonpeas stored in an environment where there is heavy *C. maculatus* infestation, a situation commonly found in many rural on-farm grain stores. The treatment was labelled PP1Ac. An EL-USB-2 data logger (Lascar electronics Inc., Pennsylvania, USA) programmed to record data every 30 min was placed in one replicate of the PICS and PP bags to record the temperature and relative humidity conditions during storage. The experiment was arranged in a randomized complete block design of the five treatments and replicated four times. All bags were kept on wooden planks in a 48 m^3 (4×4×3 m) concrete room, and stored for six months.

Oxygen and carbon dioxide concentrations in the PICS bags were measured at biweekly intervals using a Mocon Pac Check® Model 325 portable oxygen/carbon dioxide analyzer (MOCON Inc., Minneapolis, USA).

Sampling for other parameters was done on a monthly basis. To withdraw samples, bags were briefly opened and 500 g of pigeonpeas in quadruplicate were drawn using a two-inch diameter plastic tube. Each 500 g sample was analysed for moisture content before subdividing into four sub-samples of 125 g each by quartering on a flat surface. One sub-sample was used for insect damage assessment (pigeonpea seeds with one or more emergence holes were counted as damaged) and weight loss determination, another for live insect count. Moisture content determination was done with a Dickey-John mini GAC® plus moisture tester (DICKEY-john Corporation, Illinois, USA). About 400 g of grain was filled into the tester cup and the moisture content reading was recorded.

Insect damage and weight loss

Sub-samples (125 g) were sieved through a 2 mm sieve, and dust-free seeds and insect damaged and undamaged grains were separated. Weight of undamaged grains (U), weight of insect damaged seeds (D), number of undamaged seeds (Nu), and number of insect damaged seeds (Nd) were determined. Percent damage was calculated as $[Nd/(Nd + Nu)] \times 100$.

Percentage weight loss was calculated by the count and weigh method using the expression: % weight loss = $[(U \times Nd) - (D \times Nu)]/U\ (Nu + Nd) \times 100$ (Boxall, 1986). For counting live insects, sub-samples (125 g) were first kept in a refrigerator maintained at 2°C for 3 h to immobilize crawling insects. The damaged seeds were further split open to remove any insects lodged inside them. Insect counts were reported as the number of live adult *C. maculatus* per 125 g sample.

RESULTS

Storage conditions

The average temperature and relative humidity in the storage room were 24.4°C and 44.2%, respectively (Figure 1). Temperature conditions in the PICS and PP bags were generally similar throughout the storage period.

Relative humidity profiles in the PICS and PP bags differed significantly (Figure 1). In PICS bags, relative humidity remained fairly constant at about 65% in the first four months (from mid-July to mid-November), but increased steadily in the subsequent two months, reaching 68%. In the PP bags relative humidity fluctuated markedly but the general trend was one of a decline from an initial average of 64% to a final average of 58%.

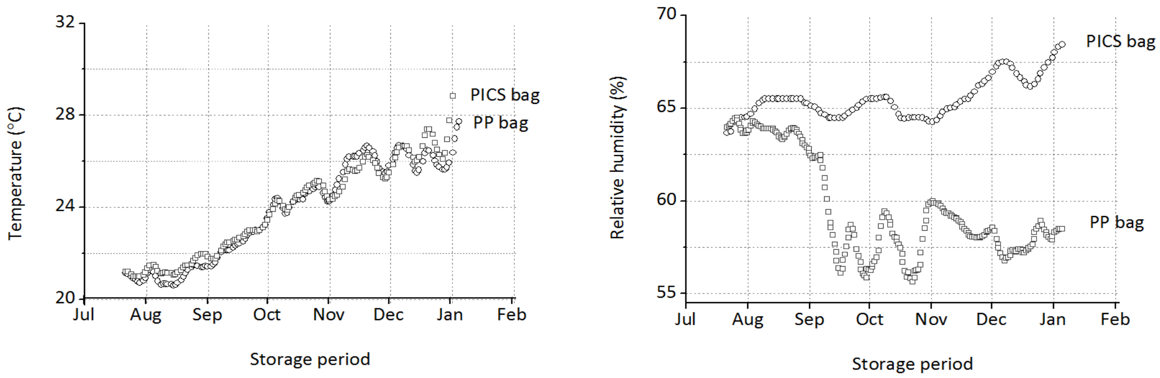

Figure 1. Temporal variations in temperature and relative humidity in PICS (□) and PP (○) bags over six months of storage.

Oxygen concentrations measured immediately after closure of the PICS bags were 20.22 and 20.51%, whereas carbon dioxide concentrations were 0.28 and 0.27% in PICS0 and PICS1, respectively (Figure 2A). When the entire storage period is viewed in perspective, changes in oxygen levels and carbon dioxide were not significantly different in PICS0 and PICS1. Oxygen concentration decreased steadily, reaching 5.19 and 4.94% in PICS0 and PICS1, respectively, after three months. By contrast, carbon dioxide levels increased gradually, levelling off at 14.50% in PICS0 and 14.62% in PICS1. Further changes in the concentrations of oxygen and carbon dioxide after the third months of storage were minimal; the final concentration of oxygen attained at sixth month was 4.71 and 4.61% in PICS0 and PICS1, respectively, whereas the respective final carbon dioxide concentrations were 14.02 and 14.44%.

Moisture content

Moisture content of pigeonpeas at the onset of storage was 12.54±0.23%. Throughout the entire storage period, the moisture content of pigeonpeas stored in PICS or PP bags differed significantly. At the end of the sixth month, moisture contents were 11.73±0.10%, 11.65±0.13%, 11.50±0.08%, 11.62±0.19% and 11.33±0.10% in PICS0, PICS1, PP0, PP1 and PP1Ac, respectively. Thus in all cases the moisture content remained below 13%, which maximizes long-term storage of pigeonpeas under stable relative humidity (70%) and temperature (25-27°C) conditions (Odogola, 1994).

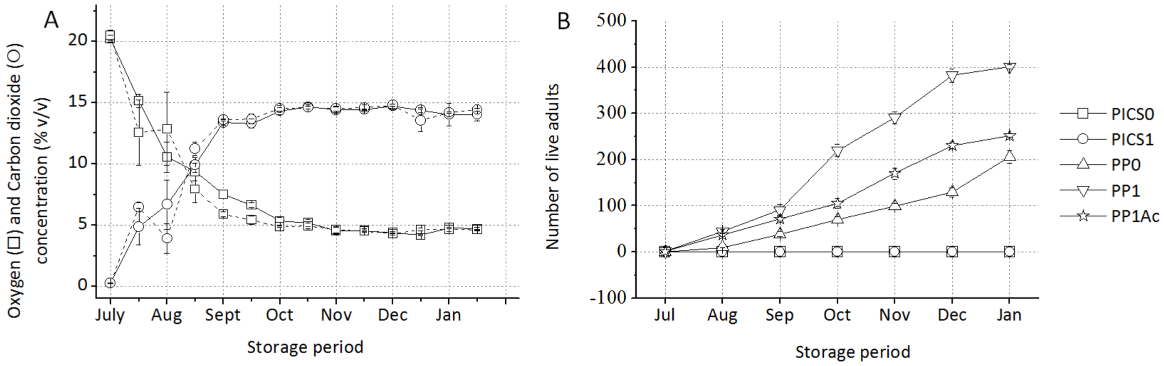

Figure 2. A. Oxygen (□) and carbon dioxide (○) levels in PICS bags filled with pigeonpea grain over a six month storage period. B. Populations (average numbers per 125 g sample) of live adult *C. maculatus* in pigeonpeas stored in PICS and PP bags for a period of six months.

Counts of living adults of *C. maculatus*

Throughout the entire storage period, there were no live adult *C. maculatus* in the PICS bags, even in PICS1 where *C maculatus* had been introduced artificially prior to storage (Figure 2B). By contrast, population of *C. maculatus* increased tremendously in the PP bags. At the outset, a substantial build up of *C. maculatus* population occurred in pigeonpeas that were stored without deliberate pre-storage infestation (PP0). Populations of *C. maculatus* also increased steadily in pigeonpeas that had been treated with Actellic Super dust (PP1Ac) although, throughout the storage period, the population remained significantly lower than the population in pigeonpeas that had not been treated with it, that is, PP1. The presence of *C. maculatus* in the PP0 bags demonstrates that re-infestation may take place from within the grain or storage environment when ordinary polypropylene bags are used for storing pigeonpeas as is commonly done by farmers. During the experiment, we observed a population explosion of *C. maculatus* in PP1 by the third month of storage, and enormous number of bruchids was seen crawling around the storage room. PICS bags, however, did not allow such invasion from outside.

Grain damage and weight loss

Pigeonpeas used in the present experiment had already been lightly damaged by bruchids when the experiments were set up, thus there was initial damage and weight loss on data collected at onset of experiment. Initial insect damage was 22.6±1.4% and the initial weight loss was 0.6±0.1% (Table 1). The damage was only minor as indicated by the low weight loss value. Throughout the entire six-month storage, insect damage and weight loss of pigeonpeas stored in PICS bags did not change (Table 1). By contrast, pigeonpeas stored in PP bags were riddled with emergence holes. Insect damage increased steadily over time, reaching 2.4, 4.2 and 3.4 times higher than the baseline damage level in PP0, PP1, and PP1Ac, respectively, after sixth months. The baseline weight losses (0.6%), on the other hand, increased by factors of 21.6, 43.6 and 22.5 reaching 13.0±0.6%, 26.2±0.4% and 13.5±0.2% in PP0, PP1 and PP1Ac, respectively, after six months. The results indicate that Actellic Super treatment can reduce grain damage and weight loss but that the insecticide would be relatively ineffective, especially if storage is done in environments that allow heavy re-infestation.

DISCUSSION

Pigeonpea is a perennial crop and harvesting takes place once a year in many growing regions and therefore the need for storage is high. The bruchid *C. maculatus* is an important pest for stored pigeonpeas (Swella and Mushobozy, 2009). One reason bruchids are important storage pests, is because of their ability to reproduce quickly in stores, together with their ability to migrate and infest clean grain stores. Because of their fear of heavy losses many pigeonpea farmers don't store their grain for long (Bett and Nguyo, 2007). Occasionally, however, they may be forced to store for protracted periods due to lack of market and harvest uncertainties (Mergeai et al, 2001). Some market-oriented farmers and traders, however, use Actellic Super as storage protectant.

Table 1. Grain damage (%) and weight loss (%) of pigeonpeas stored in PICS and PP bags.

	Storage period[1]					
	1 month	2 months	3 months	4 months	5 months	6 months
			Grain damage			
PICS1	21.9±1.8[a2]	23.4±0.9[a]	21.3±1.3[a]	23.3±1.6[a]	22.1±1.7[a]	24.3±0.3[a]
PICS0	23.3±1.7[a]	23.4±1.1[a]	23.0±0.5[a]	22.9±0.8[a]	22.3±0.9[a]	23.4±0.9[a]
PP1	31.2±2.1[b]	40.9±1.9[c]	46.3±1.7[c]	56.4±2.6[c]	72.4±2.3[d]	95.7±0.7[d]
PP0	26.2±1.9[a]	33.9±3.9[b]	40.2±2.4[b]	45.1±0.4[b]	51.9±1.9[b]	55.1±1.1[b]
PP1Ac	30.7±1.6[b]	35.8±1.1[bc]	42.3±1.1[b]	54.2±2.8[c]	59.5±3.5[c]	75.8±1.7[c]
			Weight loss			
PICS1	0.6±0.0[a]	0.7±0.0[a]	0.6±0.1[a]	0.8±0.0[a]	0.8±0.0[a]	0.7±0.0[a]
PICS0	0.7±0.0[a]	0.8±0.0[a]	0.7±0.0[a]	0.7±0.0[a]	0.7±0.0[a]	0.6±0.1[a]
PP1	6.9±1.2[c]	11.0±1.1[c]	12.5±0.3[d]	13.4±0.0[d]	13.5±0.1[d]	26.2±0.4[c]
PP0	6.2±0.2[c]	7.3±0.2[b]	10.2±0.1[b]	10.5±0.1[b]	10.5±0.1[b]	13.0±0.6[b]
PP1Ac	3.6±0.3[b]	6.8±0.1[b]	11.5±0.9[c]	11.4±0.2[c]	11.6±0.1[c]	13.5±0.2[b]

[1]Storage was conducted between July 2012 to January 2013 and initial damage before trial commenced was ca. 22%.

[2]Entries in the same column followed by same superscript letters (grain damage and weight loss) are not significantly different ($p>0.05$).

Our results show that Actellic Super did not stop the multiplication of *C. maculatus* in pigeonpeas. Some earlier papers also reported similar results, indicating possible *C. maculatus* resistance to Actellic Super (Dasbak et al., 2009; Swella and Mushobozy, 2007). This poor effectiveness is of interest because it demonstrates that the insecticide would not be appropriate especially where storage re-infestations and cross infestations can occur. Also for many farmers, prophylactic treatment of produce against insect attack is infrequent. Instead, treatment is usually prompted by detection of a live infestation (Golob, 1991). These circumstances call into doubt the usefulness of Actellic Super for preservation of pigeonpea grain. Moreover, the insecticidal potency of Actellic Super is known to diminish with time (Denloye et al., 2008), meaning that farmers have to apply the insecticide more than once where long storage periods are expected, a practice that is uneconomical and unsafe.

Storage of pigeonpeas in PICS bags prevented survival of *C. maculatus*, blocked growth of its populations and halted grain damage and dry weight losses due to the insects. A number of mechanisms are responsible for preclusion of insect survival under hermetic storage. Murdock et al. (2012) observed that decreasing oxygen and increasing carbon dioxide concentrations slow down the rate of feeding of *C. maculatus*, and at lower oxygen concentrations, feeding becomes extremely slow or even ceases. Under oxygen deprivation, oxidative metabolism is suppressed and *C. maculatus* is unable to produce metabolic water needed to support vital life processes and maintain cellular and tissue integrity. Consequently death eventually occurs due to desiccation (Murdock et al., 2012). Simultaneous exposure of insects to low oxygen and high carbon dioxide concentrations has been shown to have synergistic effect on insect mortality (Banks and Annis, 1990; Calderon and Navarro, 1980).

Losses arising from bruchid attack on stored pigeonpeas are both quantitative and qualitative. On the one hand, bruchid feeding causes quantitative losses that are characterized by loss of edible portion and loss of sellable weight. On the other hand, qualitative losses manifest in infestation and seed damage that culminates in loss of consumer appeal, market value, or complete loss of market opportunity. Some earlier studies reported practical limits beyond which insect attacks are found to cause weight loss and damage that is economically significant (Compton et al., 1998; Henckes, 1994). From the present study, storage of pigeonpeas in polypropylene (PP) bags could result in economically significant losses even with only one month of storage depending on the level of pre-storage infestation. Storage in PICS bags preserved pigeonpeas against insect attack and therefore offers a solution for such losses, while at the same time assuring consumer safety as treatment with chemical protectants would be unnecessary.

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