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Effective and economic storage of pigeonpea seed in triple layer plastic bags

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ABSTRACT

Pigeonpea [*Cajanus cajan* (L.) Millsp.] seed stored in triple layer Purdue Improved Cowpea Storage (PICS) bags for eight months retained germination and seed integrity significantly better than seed stored in traditional gunny bags. PICS bags prevented major damage caused by bruchids (*Callosobruchus maculatus* F.), while grain stored in gunny bags suffered severe losses. The aflatoxin levels in stored seed were low and not significantly different between the two storage systems. The levels of O_2 in PICS bags artificially infested with *C. maculatus* dropped rapidly during the first month of storage while the levels of CO_2 increased. Even in absence of bruchids (noninfested seed) PICS bags preserved seed germination for extended periods of time better than gunny bags; possibly due to the higher and more stable relative humidity inside the PICS bags. Higher seed germination would result in improved plant stands in the field and subsequent higher yields and increased productivity. Thus, PICS bags have shown potential to positively impact the economy of pigeonpea farmers in the semi-arid tropics.

Keywords: Bruchids; *Callosobruchus maculatus* F.; *Cajanus cajan* (L.) Millsp.; aflatoxins; seed viability; seed vigor

1. Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a short lived (3-5 years) perennial bush legume planted often as an annual crop on 4.4 million ha in sole and intercropping systems in the semi-arid tropics of Asia, Africa and Latin America (FAOSTAT, 2011). The average yield is low (755 kg/ha, FAOSTAT, 2011), but the yield potential of the new cytoplasmic-male sterile (CMS) based hybrids could reach 4 t/ha (Saxena and Nadarajan, 2010). Pigeonpea is sensitive to photoperiod and has a wide range of maturities (from super-early to long duration) (Vales et al., 2012). Pigeonpea has high protein content (21-25%, Saxena et al., 2010) and is mainly used for human consumption as dry split peas (dal), or as immature green peas (fresh or canned). Pigeonpea has a number of additional roles in subsistence agriculture: (1) after harvesting, the plants are used as fuel and construction materials; (2) most leaves drop to the ground during the crop growth period and add organic matter to the soil; (3) the roots have rhizobia that fix nitrogen (up to 40 kg/ha) and (4) help to release bound phosphorus in the soil; (5) grain and leaves are used as feed and fodder; and (6) leaves and roots have medicinal properties (Mula and Saxena, 2010).

If pigeonpea grain is to be processed as dal, farmers typically sell it to trade dealers as soon as possible after harvest, and the trade dealers then sell it to processors. Storing the grain and selling it at a time when the prices have risen due to scarcity of dal in the market or other factors could provide an economic incentive for the farmers to store; however, their need for cash at harvest time, the lack of low-cost, effective storage systems and the potential loss to storage pests deter famers from following this alternative strategy. Healthy and undamaged pigeonpea seed is needed to plant the next crop and must be properly stored by the farmers or purchased from specialist seed growers or private companies. Because of the wide range of maturities, pigeonpea seed needs to be stored for variable periods of time (up to nine months). Bruchids (*Callosobruchus* spp.) are major storage pests of pigeonpea and other legumes and cause substantial losses (Ramzan et al., 1990; Srivastava and Pant, 1989). The level of bruchid damage is affected by the original infestation level and the storage conditions. In control treatments with an initial infestation of six pairs of adult bruchids per kg of pigeonpea seed Chauhan and Ghaffar (2002) observed 91% seed damage by 41 weeks of storage, whereas with five pairs of adult bruchids in three kg of pigeonpea seed Gunewardena (2002) obtained 59% bruchid damage by six months. Bruchid damaged seed (1) has no seed value (2) sells at drastically reduced prices in the markets of any developing country and (3) is totally unfit for dal making and export.

Post-harvest losses of food grain due to insects and molds have been conservatively estimated to be 10-15% (Grolleaud, 2002) and total grain losses due to insect pests is not uncommon. The main concerns about long term storage include (1) physical damage to the seed caused by storage pests that results in weight losses and reduction of germination together with (2) additional deterioration of seed germination and quality that result from the extended storage period. Reduction of seed germination rates will result in lower plant stands and subsequent yield reduction unless the seeding rate is increased and/or reseeding practiced. These options would increase seed and/or labor cost. Storage concerns apply not only to farmers but also to breeders and seed producers who need to preserve breeder, foundation and certified seed.

Another important concern related to storage is the accumulation of secondary metabolites like aflatoxins that are toxic to humans and animals (Bryden, 2012). Bio-deterioration of pigeonpea seed in storage due to growth of fungi, especially under high humidity and warm storage conditions (i.e., Northeast Uttar Pradesh, India) causes important losses (e.g.,. decrease of shelf life of stored pigeonpea seed) (Pandey et al., 2012) and health concerns. Twenty diverse fungal species from eight genera were isolated from pigeonpea seed samples from North-east Uttar Pradesh including *Aspergillus flavus*, *A. niger* and *A. terreus* (Pandey et al., 2012). A study of aflatoxin contamination in pigeonpea samples from three agro-climatic regions of Andhra Pradesh showed no aflatoxin presence in freshly harvested samples (Rajyalakshmi 1978). After three months of storage, 20.8 per cent of the pigeonpea samples contained toxin; four per cent of these were at a level considered unsafe (above 20 μg/kg). After six months the frequency of aflatoxin contamination had further increased (Rajyalakshmi 1978). Bankole et al., (1996) detected 32.0 μg/kg of aflatoxins in pigeonpea seed stored in jute bags for six months.

A typical practice in the semi-arid tropics to protect seed from bruchid attack involves drying the freshly harvested pigeonpea seed in the sun, usually for about four days. While this may help, there is a continuing risk of post-treatment re-infestation. Dried seed is subsequently stored in metal bins, polyethylene or gunny bags and earthen structures, with turning and the application of inert dusts (mainly ash of fire wood) and neem or castor oils (Yadav, 1997). Chemical insecticides can be used to control the storage pests, but may be hazardous, especially if the farmers do not take proper precautions in choosing them and handling them. Another concern about insecticides is that they may degrade rapidly in tropical climates because of the high temperatures and humidity. Genetic resistance to bruchids could be used as a complementary way to reduce damage caused by the pest (Jadhav et al., 2012) but it takes time to develop new cultivars with high standards of yield and quality and multiple disease resistances. Solar heating combined with the use of transparent polyethylene bags prevents losses to storage pests in cowpea (Murdock and Shade, 1991, Ntoukam et al., 1997) and beans (Chinwada and Giga, 1996). Solar disinfestation was also found to be effective in controlling bruchids in pigeonpea (Chauhan and Ghaffar, 2002; Gunewardena, 2002) without negatively affect germination.

The storage procedures described above have been adopted by only a small proportion of farmers. There is a need for economically feasible, low labor intensive, safe (no use of chemicals) and convenient (easy to transport) storage technology that would benefit farmers and reduce losses due to damage caused by pests or reduction in germination and quality associated with long term storage. The triple-layer plastic bag called the Purdue Improved Cowpea Storage (PICS) system is an economic, simple, and effective technology used for cowpea and other grains; it greatly reduces losses to storage insects (Moussa et al., 2009). An intense outreach program to implement the use of PICS technology in Africa was initiated in 2007 (Baributsa et al., 2010). The PICS system might also serve as an alternative for storage of pigeonpea grain and seed. However, it is first necessary to establish that PICS hermetic bags are an effective, safe, and convenient alternative for pigeonpea storage. The objective of the present study is to evaluate the performance of the PICS storage bags versus the traditionally used gunny bags on pigeonpea (1) seed germination, (2) seed moisture, (3) seed coat color, (4) aflatoxin contamination, and (5) insect (bruchid) damage control over storage periods ranging from two to eight months.

2. Materials and methods

2.1. Seed

Around 650 kg of seed from the medium duration pigeonpea cultivar (pure line) Asha were obtained from a local farmer from Tandur, Rangareddy district, Andhra Pradesh, India. The seed was harvested on February 3rd, 2012 and naturally sun dried for four days with no chemicals applied. The following traits were evaluated at ICRISAT (Patancheru, Andhra Pradesh, India) where the storage experiment was conducted: (i) seed germination, (ii) seed moisture, (iii) weight of 100 seed, (iv) seed coat color, (v) aflatoxin levels, (vi) seed damage, (vii) numbers of adult bruchids, and (viii) numbers of eggs; the initial values found were used as the baseline reference.

2.2. Bruchids

Bruchids (*Callosobruchus maculatus* F.) were obtained from naturally field infested pigeonpea seed at ICRISAT, Patancheru, India. The population was multiplied under laboratory conditions (30 ± 2^{o}C) using seed from the medium duration pigeonpea variety Asha, the same variety as used in this study. These cultures were maintained in several plastic jars (15 cm X 10 cm diameter) covered with fine mesh lids to provide good ventilation. Based on the morphological characters of the freshly emerged adult bruchids, mating pairs were separated and shifted to the respective treatments using fine camel hair brushes. Sixty pairs of adult bruchids supplied the initial infestation in each storage bag (each bag containing 10 kg of seed) after which the bags were transferred to the seed storage area (ICRISAT, Patancheru, Andhra Pradesh, India).

2.3. Storage bags

For storage of pigeonpea we used gunny bags and PICS (Purdue Improved Cowpea Storage, http://www.ag.purdue.edu/ipia/pics/Pages/Home.aspx) bags. The gunny sacks, made from natural jute fibers, typically hold around 50 to 100 kg of seed and have high breathability, allowing air to pass through them. In order to prevent escape of adult bruchids we covered all the gunny bags with two 60 x 100 cm muslin cloth pollination bags. The PICS bags obtained from Lela Agro (Kano, Nigeria) consist of three non-connected bags (layers), the inner and middle layers composed of 80 micron high density polyethylene plastic (HDPE) while the outermost layer is woven polypropylene for strength. The storage bags used (gunny bags and PICS bags) were carefully inspected for stitching defects and sealing imperfections in order to ensure that good quality bags were used.

2.4. Experimental design

The experiment was conducted at ICRISAT, Patancheru (Andhra Pradesh, India) (17^o N, 78^o E) in a storage room at ambient temperature. The treatments combinations were: (1) gunny bags containing noninfested grain, (2) PICS bags noninfested, (3) gunny sacks infested as described above and (4) PICS bags infested with adult bruchids. Asha seed (10 kg) was placed in each bag. In the infested treatments, 60 pairs of adult bruchids were placed in the bags at the beginning of the experiment. The bruchids were gently and uniformly mixed with the seed before closing the bags. The storage bags (one layer at a time starting with the inner bag in the case of PICS bags) were closed by manually expelling the air from the bags, twisting the loose end of the bag around, folding over, and tying tightly at the base of the twist and around the folded loop using strong thread.

A total of 64 storage bags (each containing 10 kg of pigeonpea seed) were used for the experiment. A factorial experimental design was used. The fixed factors were bag type (gunny and PICS), infestation type (infested with bruchids and noninfested), and storage time (2, 4, 6 and 8 months). Each treatment combination had four replications (random factor).

2.5. Data collected: description, times, equipment and methodology used.

Data was collected upon arrival of the seed, one day before starting the experiment (on Feb. 14th, 2012) and every two months (2, 4, 6 and 8 months) for most traits except CO_2 and O_2, which were collected at various intervals (see details below). Temperature, humidity and dew points were automatically recorded every hour from the beginning to the end of the experiment using data loggers (Lascar model EL-USB-2, Whiteparish, Wiltshire, Great Britain). Aflatoxin levels were evaluated at the beginning and at the end (eight months) of the experiment.

2.6. Aflatoxins

Aflatoxin levels were measured (four replicates) before starting the storage experiment using recently harvested pigeonpea seed, and at the end of eight months of storage (in four replicates per treatment combination (1) gunny bags noninfested, (2) PICS bags noninfested, (3) gunny bags infested, and (4) PICS bags infested. A representative sample of approximately 100 g of pigeonpea seed was collected from each treatment following an indirect competitive ELISA to quantify the aflatoxins levels as described by Waliyar et al. (2005).

2.7. O_2 and CO_2 levels

Levels of O_2 and CO_2 were measured using a Mocon PAC Check® Model 325 headspace analyzer (Mocon, Minneapolis, MN, USA). Data were taken one day after initiating the storage experiment, then at short intervals during the first month and at more extended intervals until the end of the experiment (collection days: 1, 6, 16, 21, 27, 32, 48, 59, 78, 108, 184, and 247) (Figure 3). To make the gas level determinations, a circular window was cut on the surface of the outer woven layer of the PICS bags. The O_2 and CO_2 concentrations were measured around 10:00 a.m. on selected days by inserting the needle probe of the Mocon analyzer near the center of the middle and inner layers of the PICS bags; the hole in the outer bag was sealed using plastic adhesive tape; no sealing was necessary in the case of the gunny bags. Per treatment combination (gunny bags noninfested, PICS bags noninfested, gunny bags infested, and PICS bags infested) data were taken in n = 16 (first two months), n = 12 (between two and four months), n = 8 (between four and six months), and n = 4 (between six and eight months), due to the sequential removal of bags after two, four, six and eight months of storage.

2.8. Temperature, relative humidity and dew point

The data logger model EL-USB-1 (Lascar Electronics, Wiltshire, UK) was programmed to collect temperature, relative humidity and dew point information automatically every hour over the eight month storage period. One data logger was kept in the storage room under ambient conditions and four were placed inside bags, one each in the four different treatment combinations (gunny bags noninfested, PICS bags noninfested, gunny bags infested, and PICS bags infested). After finishing the storage experiment (eight months) the data loggers were removed from the bags and the temperature, relative humidity and dew point data were downloaded.

2.9. Seed damage by bruchids, number of eggs, number of adult bruchids

After two, four, six and eight months of storage, four bags per treatment combination (gunny bags noninfested, PICS bags noninfested, gunny bags infested, and PICS bags infested) were taken to an open space far from the research experimental station to avoid unintended escape of bruchids that might infest other crops. Insecticide (Nuvan - Dichlorvos- at 2 mL/L) was sprayed inside the PICS bags and inside and in between the gunny bag and protecting pollination bag in the case of the gunny bags to kill adult bruchids. The bags were closed again for 5-10 min. The seed inside the bags was thoroughly mixed. From each bag (four reps per treatment combination), two seed samples were collected: one sample of about 1 kg by volume to inspect for insects and insect damage and another sample containing >200 seeds for evaluations of seed viability and physical parameters.

The number of adult bruchids was based on a 1 kg sample per bag. The number of eggs was counted in 1000 seeds (randomly sampled from the 1 kg sample) per bag. The number of damaged seed (a seed was considered 'damaged' if one or more holes were observed) was based on 1000 seeds (the same sample used to count the number of eggs).

Seed germination, radicle and plumule length, vigor index, weight of 100 seeds and seed moisture were calculated using 100 seeds per replication (four) per treatment combination (four: gunny bags noninfested, PICS bags noninfested, gunny bags infested, and PICS bags infested) after four storage periods (two, four, six and eight months). For the germination tests, 100 seeds per replication were sterilized with 0.1% $HgCl_2$ and rinsed with distilled water, placed evenly on germination paper; the paper was rolled around the seeds, wetted with distilled water and placed in an incubator (Percival Scientific, Iowa, USA) at 25^{o} C for 10 days. Moisture was maintained by misting with distilled water daily. A seed was considered as 'germinated' if the root was at least 1 mm long; germination was expressed as a percentage. The radicle and plumule lengths of each seed were measured at 10 days using a ruler and the average value per replication per treatment per storage time was recorded. The seed vigor index was calculated as (radicle length +

plumule length) x germination percentage; this index measures the quality of the seed by combining viability of the seed and strength of the seedlings.

A separate set of 100 seeds (from the same replications, treatment combinations and storage periods as indicated above) were weighed to obtain 100-seed weight. The moisture analysis was done by the oven drying method. NMR test tubes were used to hold the seed. The seed was dried at 150^{o}C for 1 hour. Seed moisture was calculated as (initial weight - final weight)/initial weight x 100.

Seed coat color was evaluated using the Royal Horticultural Society Color Chart (RHSC). Seed coat color was recorded for four replicates per treatment combination and per storage time, including also the baseline seed coat color at the beginning for the experiment. This trait is qualitative and was used for descriptive purposes.

2.10. Data analysis

The replicated data for the different treatment combinations and storage times was entered in Microsoft Excel. SAS software (SAS, 2008) was used for the statistical analysis. Normality tests were performed (Proc Univariate). Most traits were not normally distributed (except radicle length and 100-seed weight) however we used the nontransformed data. Analysis of variance was done using Proc mixed considering bag type (gunny and PICS), infestation type (infested with bruchids and noninfested), and storage time (2, 4, 6 and 8 months) as fixed effects factors and replications as random effect factors. Phenotypic correlations (Pearson coefficients) between traits were obtained using the Proc corr statement. Comparison of means was done using protected LSD at 0.05. Graphical representations were made in Microsoft Excel.

3. Results

3.1. Baseline data of Asha seed upon arrival

The pigeonpea seed of the cultivar Asha used in the experiments was very healthy. Initial seed germination rate was high (98.8% average, SE: 0.2), and showed no evidence of pest damage (no holes and no eggs on the seeds, no adult bruchids present). The seed was dry (average 10.6% moisture, SE: 0.1), the average 100-seed weight was 8.7 g (SE: 0.2) and aflatoxins were not detected. The seed coat color was brown, RHS (Royal Horticultural Society) 175 B from the greyed-orange group.

3.2. Comparison of storage bags alone

The comparison of storage bags (gunny vs PICS), independently of infestation and storage time, indicated that on average seed germination was significantly higher in seed stored in PICS bags (88.1% seed germination in PICS versus 69.1% germination in gunny bags) (Table 1). The radicle and plumule lengths of the germinated seeds coming from PICS bags were significantly larger and the vigor index was higher (1362 vs 1003 for PICS and gunny bags, respectively) (Table 1). Seed moisture was lower in PICS bags than in gunny bags (6.5% vs. 7.2%, respectively), however 100-seed weight was comparable (Table 1). The number of adult bruchids recovered per kg of seed, the number of eggs per 100 seed and percentage of damaged seed (one or more holes) were significantly higher in gunny bags than in PICS (Table 1). The aflatoxin content was low and not significantly different between gunny and PICS bags at the end of the experiment (Table 1).

3.3. Effect of infestation alone

Bruchid infestation significantly reduced germination, radicle length, seed vigor index and 100-seed weight, but did not significantly affect plumule length and seed moisture (Table 1). As expected, the number of adult bruchids, number of eggs and seed damage was significantly higher in infested versus noninfested seed (Table 1). The level of aflatoxins at eight months was low and not significantly different between infested and noninfested conditions (Table 1).

3.4. Effect of storage time alone

The seed germination percentage dropped with the passage of time in storage (Table 1). Germination at two months and four months was acceptably high, whereas germination at six and eight months was below the accepted 75% germination threshold set by the seed certification standards. Radicle length was not affected by storage time for six months (8 - 8.3 cm), but it was significantly reduced (7.5 cm) by eight months (Table 1). On the other hand, plumule length reached a peak (maximum length) at six months (Table 1). The seed vigor index was significantly highest at two months of storage (1292) and significantly lowest at eight months (1056) (Table 1). The lowest seed moisture was achieved at two months of storage; however the lowest 100-seed weight was obtained at eight months probably due to mass loss caused by infestation. Seed damage increased over time, however, the number of adult bruchids and eggs per seed were not significantly different over the storage period probably indicating population size equilibrium. Seed germination and seed vigor index were negatively correlated with traits associated with pest traits (adult bruchids, eggs and seed damage) (Table 2),

3.5. Comparison between treatments combining storage bag type and infestation

In general, seed germination dropped in storage over time in all treatment combinations (Fig. 1A). Seed germination remained high during the storage period in the case of noninfested seed kept in PICS bags (reaching 92.2% germination at eight months of storage) and in PICS bags containing infested seed, which had 77.0% germination at eight months of storage; these values are within the acceptable limits (>75% established by the seed industry) (Fig. 1A). On the other hand, noninfested seed stored in gunny bags only maintained acceptable seed germination (>75%) for four months, and infested seed from gunny bags was not acceptable for use as seed after storing it for only two months because germination was reduced to 68.8% (Fig. 1A). There was also a reduction of seed vigor over time in all the treatment combinations (Fig. 1B). The vigor index of seed stored for eight months in PICS bags containing noninfested and infested seed was high (1368 and 1259 for PICS noninfested and infested, respectively), whereas seed vigor of noninfested seeds stored in gunny noninfested bags was near intermediate (1046) and infested seed stored in gunny infested bags had a significantly lower vigor index (549) (Fig. 1B) coinciding also with the lowest germination (44.5%) (Fig. 1A) and shortest radicles and plumules (6.4 mm and 5.9 mm, respectively, data not shown in figures).

Seed moisture dropped dramatically in all treatment combinations during the first two months of storage (from 10.6% at the beginning of the experiment to an average of 5.4% across treatments by two months) but subsequently increased at four months (7.7% average across treatments) (Fig. 1C) probably due to increase in ambient relative humidity by the beginning of the rainy season. There were no significant differences between treatments for seed moisture at two and four months after storage (Fig. 1C). By six and eight months of storage, the moisture of seed stored in PICS bags (noninfested and infested) was lower than in seed from gunny bags (noninfested and infested), with PICS noninfested seed having significantly the lowest (6.0%)

and infested seed in gunny bags significantly the highest (7.7%) (Fig. 1C). There was not much difference between treatments for 100-seed weights, but infested seed from gunny bags had consistently lower seed weights (around 0.6 g less weight) than the other treatments probably due to loss of mass caused by bruchid damage. At eight months of storage, seed weights from gunny bags containing infested seeds was 7.1 g whereas the other treatments ranged from 8.0-8.3 g) (Fig. 1C). The pigeonpea seed coat color after harvesting was RSH 175 B (from the greyed-orange group) and got darker over time in the case of noninfested gunny (175 A by eight months) and PIC bags (175 A by six months and 166 A by eight months), on the other hand, the seed coat color of infested gunny and PICS bags did not became as dark by eight months (166 B).

The number of adult bruchids per kg of seed reached the highest levels inside infested gunny bags stored for 2 months (879.8 adult bruchids per kg); the size of the population of adult bruchids rose and fell at the following storage times (four, six and eight months), reaching 641.8 adult bruchids per kg at eight months of storage (Fig. 2A). The PICS infested treatments showed a slow but linear increase of 14.6 adults per month ($y= 14.6x$, R^2 0.96), the values were very low and not significantly different from those of noninfested treatments of gunny and PICS bags for six months, but at eight months there were significantly higher number of adult bruchids (112.0 adult bruchids per kg of seed) inside infested PICS bags than the noninfested treatments (8.6 and 4.3 adult bruchids per kg of seed for noninfested gunny and PICS respectively) (Fig. 2A). The number of eggs per 100 seeds in infested gunny bags was similar at the different data collection times (two, four, six, and eight months of storage); the highest values were 67.4 eggs per 100 seed at six months of storage (Fig. 2B). The number of eggs per 100 seeds in the PICS infested treatments at four months was ten times lower than in gunny infested but significantly higher

(6.8 eggs per 100 seeds) than in the noninfested treatments; the values at the other storage times (two, six and eight months) for gunny noninfested and PICS (infested and noninfested) were low and not significantly different (Fig. 2A). The percentage of seeds damaged increased rapidly during the first four months in the case of infested gunny bags and the increase continued during the following months (six to eight) although at a slower pace, reaching 16.8% at eight months of storage (Fig. 2C). The trend in percentage of damaged seed in PICS infested bags was very similar to the trend observed for eggs per 100 seed but the values were low (0.7% seed damaged in PICS infested at 8 months) and non-significantly different from those observed in noninfested PICS and gunny bags (Fig. 2C). We should point out that a seed was considered 'damaged' if a minimum of one hole was observed, in most cases only one hole was observed per seed but in some cases more than one hole were observed. The levels of aflatoxins were low and not significantly different between the different treatment combinations at eight months of storage (Fig. 2D).

Noninfested gunny bags had similar levels of O_2 as did infested gunny bags, hovering around 20%, at all storage time periods evaluated, except at 27 days when the level of O_2 in gunny infested bags was significantly lower (19.4%) than in gunny noninfested bags (Fig. 3A). PICS infested bags had significantly lower O_2 than the other treatments at most times evaluated reaching the lowest level at 21 days of storage (6.2% O_2) (Fig. 3A). The levels of O_2 in PICS infested bags recovered gradually thereafter, reaching similar levels of O_2 as PICS noninfested bags at 184 days of storage (Fig. 3A). The levels of O_2 in PICS infested bags dropped again at eight months of storage (247 days) (up to 14.2%) but there were no significant differences between treatments (high SE for PICS infested) (Fig. 3A). Levels of CO_2 were inversely proportional to the levels of O_2 in all treatments (Fig. 3A and 3B). The levels of CO_2 in PICS infested bags were significantly higher than in the other treatments, followed by PICS noninfested bags and gunny bags (infested and noninfested) (Fig. 3B). The CO_2 content of infested gunny bags was similar to noninfested gunny bags (Fig. 3B). CO_2 levels inside PICS infested bags increased rapidly during the first month of storage (peak at 21 days of storage, CO_2 6.2%), and dropped afterwards for the next six months; at eight months, a CO_2 increase was observed (2.6%) however at 184 and 247 days (eight months) no significant differences were observed between treatments (Fig. 3B). The increase in CO_2 and decrease in O_2 by the end of the experiment in PICS infested bags may be explained by an increase in insect activity (more adult bruchids present) (Fig. 3B, Fig. 2A) or vice versa.

3.6. Fluctuations in temperature, relative humidity, and dew point

The data loggers measured temperature, relative humidity and dew point inside each treatment combination (gunny noninfested, PICS noninfested, gunny infested and PICS infested), every hour (data plotted based on Fig. 4 represent daily averages), but since only one data logger was present in each treatment combination, the comparisons do not have statistical power and should only be considered as trends.

Temperatures inside gunny and PICS bags were similar over time for gunny noninfested, PICS noninfested, gunny infested and PICS infested, respectively (Fig. 4A, Fig. 4B) and fluctuated in parallel with the ambient temperature (Fig. 4C). There were some unexpected deviations when the temperature inside the gunny bags was higher than in PICS bags: there was a one peak increase of temperature inside gunny noninfested bags at the beginning of the third month of storage and another increase in temperature (two peaks) between the end of July and the end of August (Fig. 4A); in gunny infested bags the temperature started increasing one month after storage and remained higher than in PICS noninfested for approximately one month (showing two peaks) (Fig. 4B). The observed increases in temperature may correspond to increases in bruchid population growth. The peak increases in temperature observed in noninfested and infested gunny bags in comparison with PICS bags were paralleled by the dew points and were mirror images of the relative humidity of the corresponding treatment combination (Fig. 4A, Fig. 4B). The relative humidity inside PICS bags was more stable over time than in gunny bags; in the later the relative humidity fluctuated roughly in parallel with the ambient relative humidity (Fig. 4A, Fig. 4B, Fig. 4C, Fig. 4D). When comparing noninfested versus infested PICS bags, the relative humidity of noninfested PICS was on average 8.2% higher than in PICS infested (60.2 and 52.0%, r.h. respectively), whereas the difference between noninfested and infested gunny bags (43.8 and 46.0%, respectively) was only 2.2% higher in infested gunny bags (Fig. 4A, Fig. 4B). Dew points inside noninfested and infested gunny bags were similar (average of 19.1 and 19.9^{o}C, respectively), whereas in PICS bags the average was 23.7^{o}C and 21.7^{o}C for noninfested and infested conditions, respectively.

4.0. Discussion

PICS bags control bruchid reproduction and damage in cowpeas and other crops (Baributsa et al., 2010, Murdock et al., 2012, Baoua et al., 2012); our study indicates that this is also true in the case of another legume, pigeonpea. We confirmed this by determining the number of adult bruchids per kg of seed, number of eggs and number of damaged seed per 100 seeds inside gunny and PICS bags using noninfested and artificially infested pigeonpea seed and storing it for up to eight months. In PICS bags containing infested seed, the damage level was only 0.7% after eight months of storage compared with 16.8% damage in infested gunny bags (Fig. 2C). The seed damage we observed in gunny bags by eight months was not extremely high, but there were nearly 60 eggs per 100 seeds (Fig. 2B) and more serious damage would be expected if the storage period were further extended. The fact that we covered the gunny bags with two pollination bags to avoid escape of adult bruchids could have restricted exchange of gases and might have partially inhibited the reproduction of adult bruchids in comparison with gunny bags alone. In control treatments with five pairs of adult bruchids per three kg of pigeonpea seed Gunewardena (2002) obtained 59% seed damage by six months. In a similar study (Chauhan and Ghaffar, 2002), the control treatments with an initial infestation of six pairs of adult bruchids per kg (similar to our study, 60 pair of bruchids per 10 kg) had 91% seed damage by 41 weeks of storage. Thus, the level of bruchid damage in unprotected pigeonpea can be be very high.

The levels of O_2 inside the PICS infested bags dropped rapidly during the first month of storage and the levels of CO_2 increased (Fig. 3A, Fig. 3B); this negatively affected the life cycle of the bruchids and resulted in their control. The effectiveness of hermetic storage for preserving grains against insect pests has long been connected with the depletion of oxygen and parallel rise in carbon dioxide (see review by Navarro, 1994) and suppression of population expansion has been directly linked to inadequate supply of water resulting from an inadequate supply of O_2 so the insects eventually die by desiccation (Murdock et al., 2012).

Seed germination and seed vigor index were negatively correlated with traits associated with pest traits (adult bruchids, eggs and seed damage) (Table 2). Since PICS bags arrest pest damage we observed a substantial benefit in the form of preservation of seed germination and the seed vigor index (Fig. 2A, Fig. 2B, Fig. 2C, Fig 1A, Fig. 1B). In addition, seed germination rate and seed vigor index were better over time in PICS bags than in gunny bags even when the seed was not infested (Fig. 2A, Fig. 2B, Fig. 2C, Fig 1A, Fig. 1B). This may have been due, in part, to the higher and more stable relative humidity inside the PICS bags. This is a very important finding; using PICS bags would allow farmers to store seed for extended periods of time and gain flexibility to take seed to the market at a time when they could get higher prices. It would also allow them to save seed for the next planting season (or sell seed) because acceptable germination rates are obtained (>75%). This would ensure an acceptable initial plant stand in the field that would lead to higher yields. The reduction of O_2 and increase in CO_2 in PICS infested bags did not reduce seed germination and vigor (Fig. 4B, Fig. 1A). We have not tested the processing quality of pigeonpea grain stored in PICS bags, but we believe there should not be any negative effects. Banks (1981) indicated that for low moisture content grain, low O_2 and high CO_2 concentrations do not have detrimental effect on germination, milling and baking properties of wheat, or organoleptic properties of rice, though for intermediate and high moisture grains, quality is affected (Moreno et al, 1988).

The pigeonpea seed used was acceptably dry (10%) at the beginning of the experiment and the seed moisture was reduced during storage (greatest reduction by 2 months, and 6.6 - 7.7% by eight months) (Fig. 1C). Aflatoxins were not detected in the seed at the beginning of the experiment; this agrees with the findings by Rajyalakshmi (1978). Other crops having much higher moisture levels at harvest or soon after harvest could be problematic if the seed is stored in hermetic plastic bags because the environment inside the bag has high relative humidity and together with warm temperatures would favor the development of fungi and production of toxins. The United States Food and Drug Administration (FDA) has used a 20 ppb (µg/kg) aflatoxin tolerance for human-consumed agricultural commodities almost since the beginning of the mycotoxin regulatory program; European regulations are much stricter (Robens and Cardwell, 2005). The levels of aflatoxin detected by the end of the present storage experiments were very low and not significantly different between the bag systems and treatment combinations (Fig. 2D). Bankole et al., (1996) detected levels of aflatoxins above the permissive levels in pigeonpea seed stored in jute bags by six months (32.0 µg/kg aflatoxins) and by eight months (21.3 µg/kg) in iron bins. The seed moisture at the beginning of the experiment in Bankole et al., (1996) was higher than in our case (13.6% vs 10.6%) and the environmental conditions were also different (Nigeria vs India). Prevention strategies to reduce the impact of mycotoxins in maize have been reported by Chulze (2010), and similar recommendations apply to pigeonpea. Pigeonpea grain/seed should be stored as dry as possible. The safe maximum moisture content for storing pigeonpea grain corresponds to 13% (at equilibrium with air at 70% relative humidity) and 1% less (12%) is recommended in the case of seed (Odogola, 1994). The grain/seed should also be as clean, damage free (by insects, and by harvest or post-harvest handling) and as insect free as possible before initiating storage in order to minimize the risk of fungal growth and aflatoxin contamination during storage.

In India, there are several categories of pigeonpea seed (Saxena 2006) and the price varies accordingly (personal communication by Sameer Kumar, ICRISAT, Patancheru, India). The price of breeder's seed is fixed by the government every year; for the year 2011-12 the price was Rs. 90 per kg (1 Dollar = 54.8 Rupees, April 4, 2013). The foundation seed price is fixed by individual states; for Andhra Pradesh (i.e.) the price for 2011-12 was Rs. 75 per kg. The price of truthfully labeled seed of varieties is set by private seed companies, and is usually around 70-100 Rs. Per kg. The price of certified seed for hybrids (A x R) is determined by the seed companies; in 2011-12 it ranged from Rs. 150 to Rs 200 per kg. For pigeonpea grain, the procurement price is subject to the minimum support price (MSP) fixed by the government of India; this was 3200

Rs. per 100 kg for 2011-12. The market price of grain should be higher than the MSP, usually around 35 to 45 Rs. more per kg, but it depends on demand at a given local market.

The use of PICS bags to store pigeonpea seed and/or grain would likely provide direct and indirect economic benefits. The germination percentage of seed stored in PICS will be higher than those stored in gunny bags due to less pest damage and better preservation of the germination capacity over time; this will be more relevant when storing seed for extended periods as would be the case for extra-short and super-early pigeonpea varieties. Higher germination will result in better plant stands and higher yields which represent an indirect economic advantage of using PICS bags. Other indirect cost savings (seed and labor) would result from being able to use lower seed planting rates and/or by not reseeding In the case of pigeonpea grain the economic advantage would come from preserving the grain until the market prices become more competitive and/or until there is scarcity of grain in the market.

The price of the PICS bags is relatively affordable (at approximately US$ 3), especially if manufactured and distributed directly in the country where they are used. Manufacturing PICS bags in the country where pigeonpea is produced would also stimulate the local plastic manufacturing industry. In addition, the PICS bags can be re-used and preserve (high quality, insect free) cowpea seed equally well (Baoua et al., 2012) for a second and third year, substantially increasing the return on the investment. Farmers might be tempted to use a single layer plastic bag to save money, however this is not recommended. The two plastic liners of the PICS system serve as a safeguard to preserve proper airtight conditions if one of the bags is compromised (physical damage to the outer layers from wear and tear or perforations to the inner layer made by insects). This was confirmed by Baoua et al., (2013b) when comparing two versus one plastic liner (within the PICS system) and by Baoua et al. (2013a) when comparing PICS (two plastic liners) versus the commercially available GrainPro SuperGrainbags™ (similar to the PICS but with only one highly oxygen impermeable plastic liner) (GrainPro, Concord, MA, USA). De Groote et al. (2013) indicated that SuperGrainbags™ were effective in controlling pests in maize in Kenya but not all insects died and all the liner bags of the SuperGrain™ bags were perforated in the experiments conducted. Comparing one versus two plastic liners in the PICS system Baoua et al. (2013b) indicated that the concentration of O_2 was significantly lower if two plastic liners were used (i.e., by day 19, the O_2 concentration was 10.5±1.2% versus 15.3±1.5% for the two and one liner bags, respectively) thus, if two plastic liners are used (triple bag PICS system) there is less O_2 for insect respiration.

The initial cost of the PICS and SuperGrainbags™ is probably comparable (US$3-4, depending on the geographic location); however, since the PICS can be more confidently reused (after careful inspection and replacement of the inner bag if holes are present) the net cost should be lower in the case of PICS bags. GrainPro is working to improve the SuperGrainbags™ with new models that are more resistant to insect penetration and also offer the option of inserting oxygen absorbers inside the bags to more efficiently reduce the oxygen concentration (http://www.grainpro.com/?page=grainpro-supergrainbag-4r); in addition, very strong single layer bags that can be used as stand-alone (without the jute bag) will be released soon (http://www.grainpro.com/?page=grainpro-supergrainbag-forte). Larger structures for hermetic storage of grain are available (i.e. metal bins, cocoons™, etc.) but may or may not be suitable, affordable or available for small farmers over very large areas. A well-performing technology represents only one step toward benefitting low-resource farmers. Those farmers must know about the technology, must express a demand for it, and it must be in easy geographic reach as well as affordable in a sustainable way. Farmers should explore the availability and affordability of alternative hermetic storage system in their geographic location in order to select the type that works best for their circumstances.

One sentence summary

Storing dry pigeonpea [*Cajanus cajan* (L.) Millsp.] grain/seed in PICS (Purdue Improved Cowpea Storage) bags offer a cost effective option for farmers in the semi-arid tropics, farmers enabling them to preserve their own high quality seed (good viability, low aflatoxin contamination and low bruchid - *Callosobruchus maculatus* F. - damage) and to store dry whole grain (for dal making) for extended periods of time in order to obtain higher market prices.

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Figure legends

Figure 1. Bi-monthly means of germination percentage (A), vigor index (B), moisture (C) and 100-seed weight (D) of pigeonpea stored in treatment combinations of noninfested and infested seed stored in gunny and PICS bags for a total period of eight months. Within each time period, treatments sharing a letter were not significantly different at probability 0.05.

Figure 2. Bi-monthly means of the number adult bruchids per kg of seed (A), number of eggs per 100 seed (B) and percent damaged (C); and aflatoxin content (D, determined only at the beginning of the experiment and at the end -eight months-) of seed stored in treatment combinations of noninfested and infested seed stored in gunny and PICS bags for a total period of eight months. Within each time period, treatments sharing a letter were not significantly different at probability 0.05.

Figure 3. Oxygen (A) and carbon dioxide levels (B) inside bags representing treatment combinations of noninfested and infested seed stored in gunny and PICS bags for a period of eight months. Within each time period, treatments sharing a letter were not significantly different at probability 0.05.

Figure 4. Temperature, dew point, and relative humidity inside gunny and PICS bags containing noninfested (A) and infested (B) seed and temperature and relative humidity outside (C).

Tables

Table 1. Means of traits evaluated in the pigeonpea storage experiment based on storage bag type (average over eight months of storage), bruchid infestation condition (average over eight months of storage) and storage time.

	Germination	Radicle length	Plumule length	Seed vigor index	Seed moisture	100-seed weight	Adult bruchids/kg seed	Eggs/100 seed	Seed damage	Aflatoxins (8 months of storage)
	%	cm	cm		%	g	no	no	%	ppb
Bag type										
Gunny	69.1	7.7	6.6	1003.3	7.2	8.2	394.0	32.0	7.3	1.10
PICS	88.1	8.3	7.2	1362.0	6.5	8.4	38.7	2.0	0.5	0.68
Mean	78.6	8.0	6.9	1182.7	6.9	8.3	216.4	17.0	3.9	0.89
LSD (0.05)	1.7	0.3	0.4	56.0	0.5	0.2	63.5	4.4	0.9	NS
Infestation										
No	87.1	8.3	6.9	1325.8	6.8	8.6	3.0	0.5	0.3	0.39
Yes	70.2	7.7	6.9	1039.5	7.0	8.0	429.7	33.4	7.6	1.39
Mean	78.7	8.0	6.9	1182.7	6.9	8.3	216.4	17.0	4.0	0.89

LSD (0.05)	1.7	0.3	NS	55.9	NS	0.2	63.5	4.4	0.9	NS
Storage										
2 months	87.9	8.3	6.3	1292.1	5.4	8.1	229.7	16.1	2.4	
4 months	81.1	8.3	6.5	1203.2	7.7	8.7	207.6	17.3	4.4	
6 months	73.8	8.0	8.0	1179.9	7.3	8.5	236.5	17.8	4.3	
8 months	71.9	7.5	7.0	1055.6	7.1	7.8	191.7	16.7	4.6	
Mean	78.7	8.0	7.0	1182.7	6.9	8.3	216.4	17.0	3.9	
LSD (0.05)	2.4	0.4	0.6	79.1	0.7	0.2	NS	NS	1.3	

Table 2. Pearson correlation matrix for traits measured in the pigeonpea storage experiment.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) Germination	1.00								
(2) Radicle length	0.68	1.00							
	<.0001								
(3) Plumule length	**0.03**[a]	**0.10**	1.00						
	0.82	***0.43***							
(4) Seed vigor index	0.92	0.77	0.36	1.00					
	<.0001	*<.0001*	*0.00*						
(5) Seed moisture	-0.36	-0.31	**0.19**	-0.28	1.00				
	0.00	*0.01*	***0.13***	*0.03*					
(6) 100-seed weight	0.51	0.42	0.20	0.54	**0.21**	1.00			
	<.0001	*0.00*	*0.11*	*<.0001*	***0.09***				
(7) Adult bruchids	-0.76	-0.37	**-0.23**	-0.75	**0.04**	-0.50	1.00		
	<.0001	*0.00*	***0.07***	*<.0001*	***0.75***	*<.0001*			
(8) Eggs	-0.80	-0.46	-0.30	-0.81	**0.14**	-0.47	0.90	1.00	

	<.0001	*0.00*	*0.02*	*<.0001*	***0.27***	*<.0001*	*<.0001*		
(9) Seed damage	-0.84	-0.47	-0.28	-0.83	**0.21**	-0.48	0.87	0.91	1.00
	<.0001	*<.0001*	*0.03*	*<.0001*	***0.10***	*<.0001*	*<.0001*	*<.0001*	

[a] Bold font: NS (the *P* values are shown in italics).

Figures

Fig. 1.

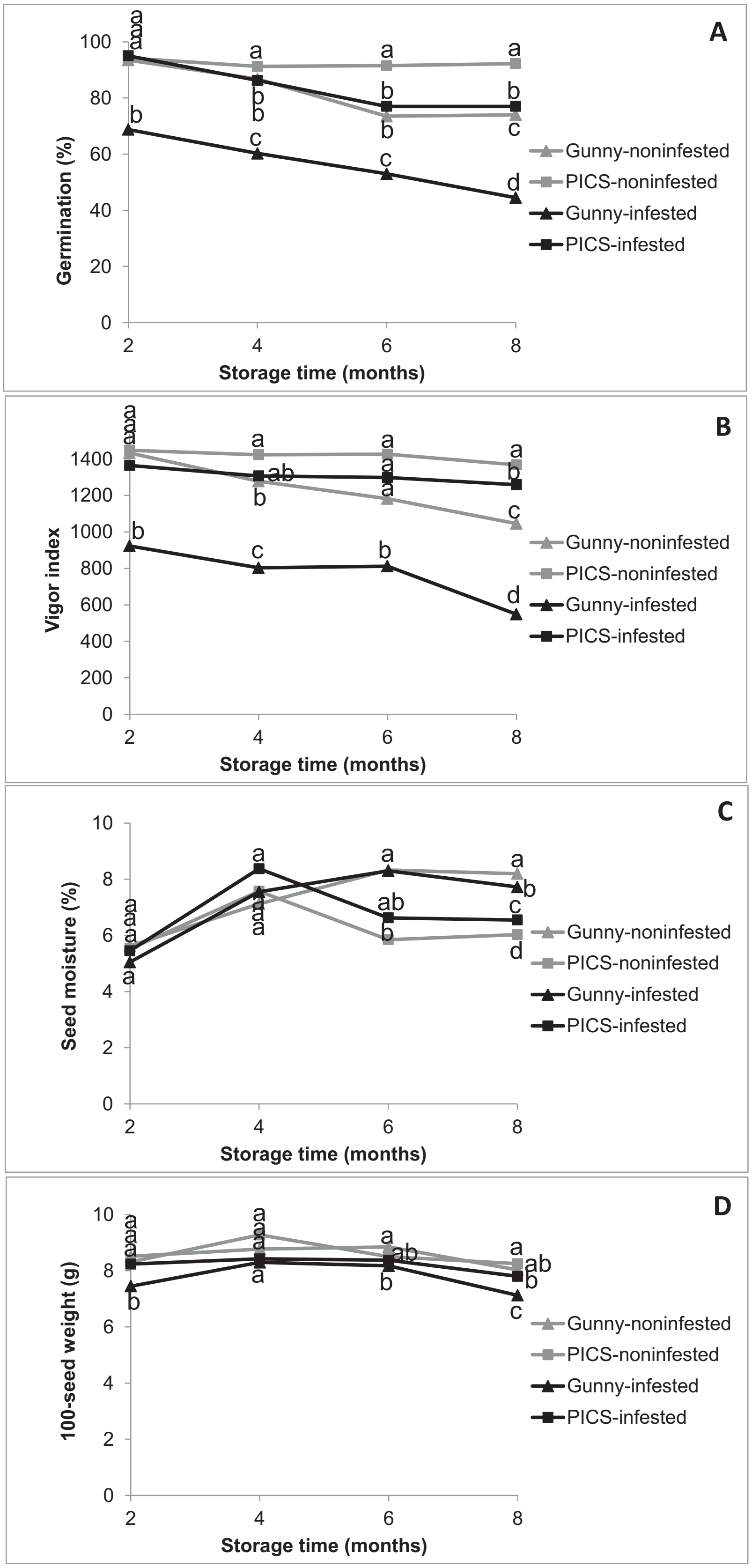


Fig. 2.

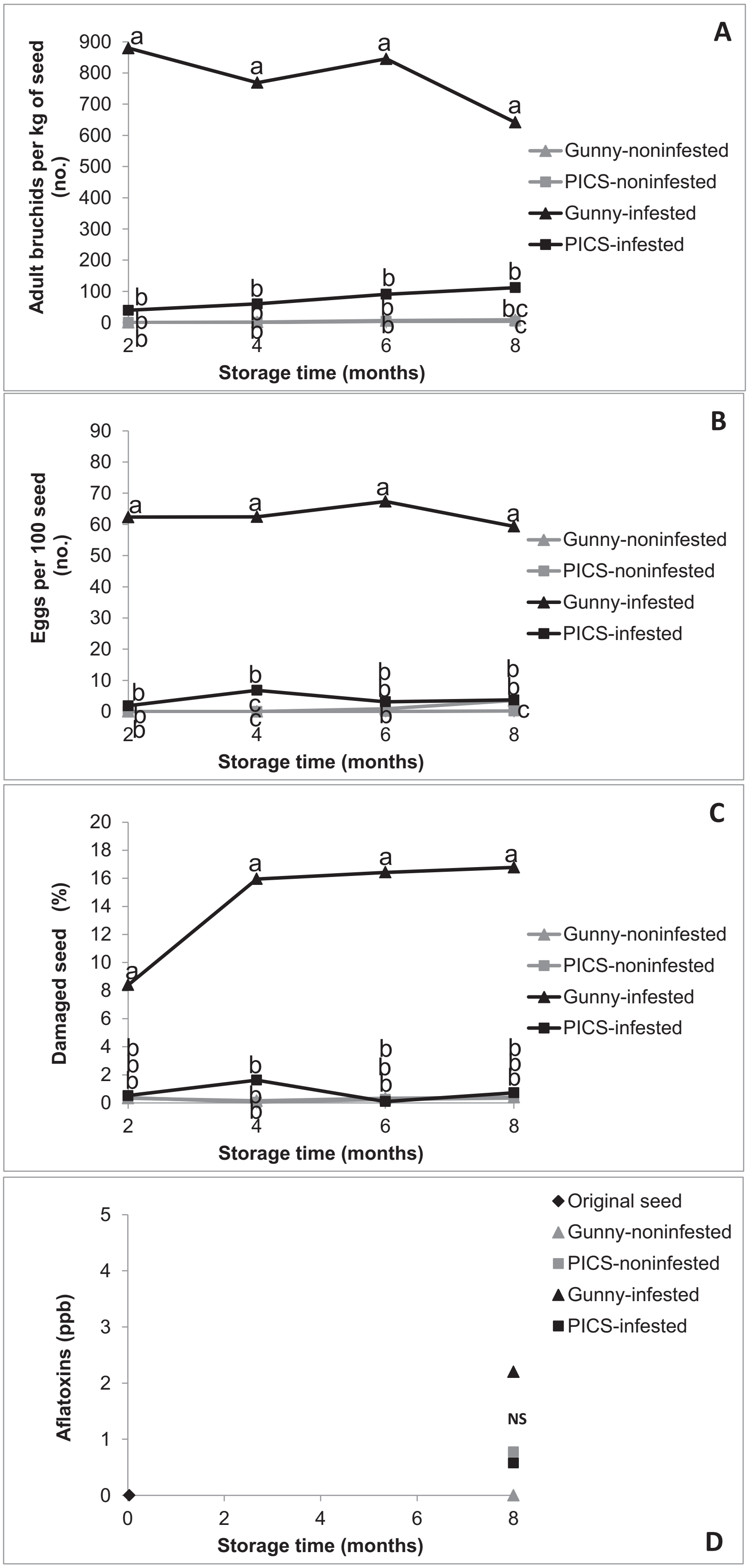
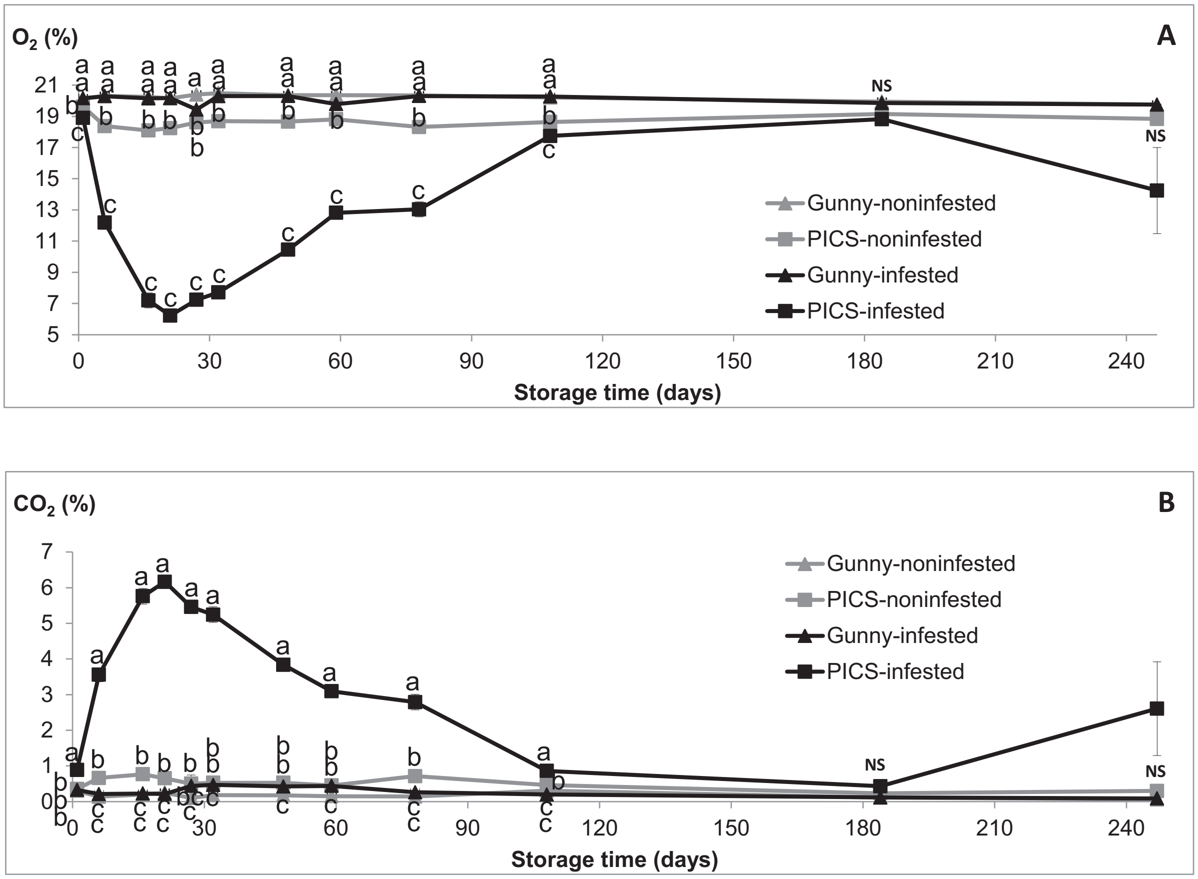
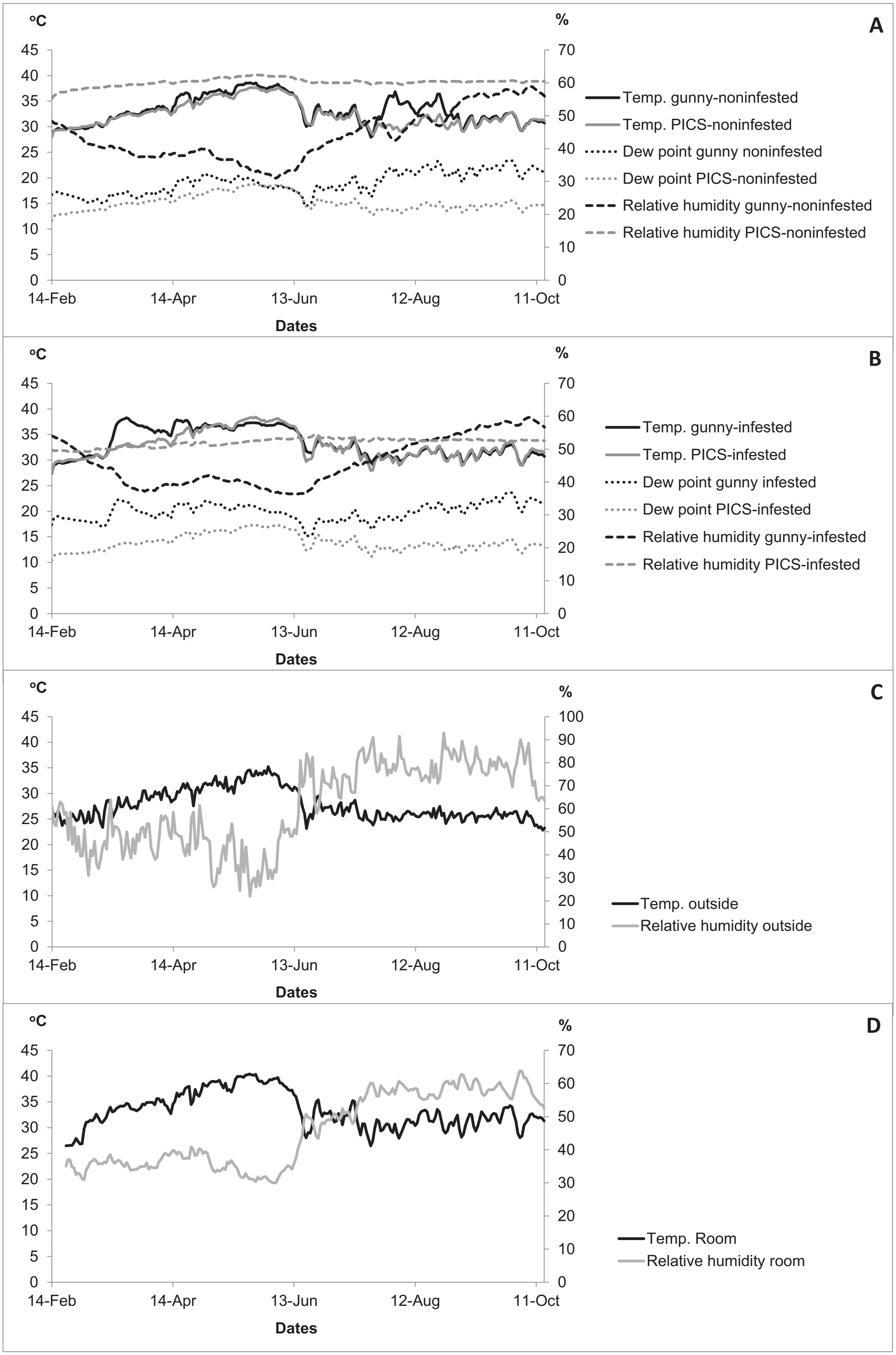

A
Adult bruchids per kg of seed (no.)
900
800
700
600
500
400
300
200
100
0
2
4
6
8
Storage time (months)
Gunny-noninfested
PICS-noninfested
Gunny-infested
PICS-infested
B
Eggs per 100 seed (no.)
90
80
70
60
50
40
30
20
10
0
Storage time (months)
C
Damaged seed (%)
20
18
16
14
12
10
8
6
4
2
0
Storage time (months)
D
Aflatoxins (ppb)
Original seed
NS
5
4
3
2
1
0
Storage time (months)

Fig. 3.



O2 (%)
A
Gunny-noninfested
PICS-noninfested
Gunny-infested
PICS-infested
Storage time (days)
NS
CO2 (%)
B
Gunny-noninfested
PICS-noninfested
Gunny-infested
PICS-infested
Storage time (days)
NS

Fig. 4.



A
ºC
%
Temp. gunny-noninfested
Temp. PICS-noninfested
Dew point gunny noninfested
Dew point PICS-noninfested
Relative humidity gunny-noninfested
Relative humidity PICS-noninfested
14-Feb
14-Apr
13-Jun
12-Aug
11-Oct
Dates
B
Temp. gunny-infested
Temp. PICS-infested
Dew point gunny infested
Dew point PICS-infested
Relative humidity gunny-infested
Relative humidity PICS-infested
C
Temp. outside
Relative humidity outside
D
Temp. Room
Relative humidity room