

## Full Length Research Paper

# Are nonwoven synthetic pollination bags a better choice for sorghum breeding?

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This work investigated the effects of seven pollination bag treatments on three varieties of sorghum for: grain loss to birds; total weight of five panicles (g); total grain weight of five panicles (g); average grain weight per panicle (g); germination per cent; and occurrence of grain mold during 2016. Varieties were: 1167048 hybrid (brown seeded); BR007B (red seeded); and P9401 (white seeded). The bag treatments were: 1. No bagging; 2. Kraft paper; 3. Kraft paper + plastic bag screen; 4. Used duraweb® SG1; 5. Used duraweb® SG2; 6. New duraweb® SG1; 7. New duraweb® SG2. High bird pressure resulted in 100% seed loss on uncovered panicles and 75% under Kraft paper pollination bags. Birds preferred white seeded P9401, which led to no seed recovery under Kraft paper bags. There was virtually no bird damage with all other pollination bags. For panicle and grain yields the varieties performed in the order 1167048>BR007B>P9401. Unprotected panicles and paper bag treatments had the lowest yields. Panicles covered with the new synthetic bags exhibited 195 to 652% higher yields compared to Kraft paper bags. Varieties x bag type interactions were not important as they contributed 4 to 6% to the total sum of squares for yield traits. Germination test under normal and stress conditions showed no significant adverse effect of bag treatments on seed health. Reused bags performed as well as new bags for all of these traits. Varieties differed significantly for the occurrence of five grain mold pathogens, with highest occurrence of *Alternaria*, up to 40%, on 1167048 hybrid. Of the five pathogens, bag types differed significantly for *Phoma* with the highest occurrence of 9% on re-used duraweb®SG2 bags. Thus bags require disinfecting and cleaning before re-use. It is concluded that nonwoven synthetic bags are a better choice than the Kraft paper pollination bags for increasing the grain yield and virtually eliminating the bird damage in sorghum.

**Key words:** Sorghum, nonwoven fabrics, kraft paper, pollination bags, bird control, grain mold.

## INTRODUCTION

This study reports the results of a follow up study from that of Schaffert et al. (2016) on novel pollination bags for

the outcome of seed harvest in sorghum. Experiments by them in 2015 showed the superiority of pollination bag

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made of nonwoven fabrics over the control Kraft paper bags in many respects. It was suggested that synthetic nonwoven bags may be re-used within the same or different seasons (Hayes and Virk, 2016) but there was no experimental evidence to support this in sorghum.

Therefore, in the present experiment, two treatments of nonwoven pollination bags saved and re-used from the 2015 experiments were included to test whether they could be reused. Since 2016 had higher bird pressure than 2015 at Sete Lagoas (Brazil) the comparison of seed harvest over two years allowed verification of the strength of new and used nonwoven bags for their bird resistance. In addition, the present investigation included the quantification of the occurrence of five grain mold causing pathogens under different types of bags. The present study extends our knowledge of the influence of different pollination bag fabrics on seed harvest and increasing the awareness of plant breeders in general, and sorghum breeders in particular, that the choice of pollination bags could be an important factor in improving the efficiency of plant breeding. The replacement of traditional paper pollination bags by those made from novel nonwoven fabrics could result in better seed harvest (Adhikari et al., 2014; Gaddameedi et al., 2017; Gitz et al., 2013, 2015; Schaffert et al., 2016; Vogel et al., 2014).

This work lays a foundation for a new research area of developing and testing new nonwoven fabrics for the pollination bags that provide a micro-environment closer to ambient than paper bags, for healthy seed development. The objectives of the present study on sorghum were to: 1. Confirm the efficacy of nonwoven pollination bags over another year with contrasting bird pressure; 2. Assess the relative occurrence of seed borne diseases within pollination bags; and 3. Test the reusability of pollination bags made from the synthetic fabrics.

## MATERIALS AND METHODS

The present investigation was carried at the Embrapa Milho e Sorgo Research Station in Sete Lagoas, Minas Gerais, Brazil during the 2016 normal sorghum growing season (date of sowing 20th April and date harvesting 9th September). EMBRAPA is the National Maize and Sorghum Research Center of Brazilian Enterprise for Agriculture Research which coordinates all sorghum research in Brazil. The experiment was conducted in a split-plot design with three varieties in the main plots, and seven bag type treatments in the sub-plots in four complete replicate blocks. Of the 7 rows of a variety whole-plot in a replicate block, one row was allocated to each of the 7 bag treatments. A sub-plot consisted of one five meter long row having 8 to 10 plants per meter.

The spacing between rows was 70 cm. Two border rows were provided after every main plot in any replication. Five panicles were covered with a pollination bag treatment just when they had started emerging from the flag leaves before natural pollination. Bags on individual plants were applied before anthesis. As varieties differed in time of flowering, bags applied to panicles of different varieties were at different times within a period of about two weeks; P9401 was the earliest to flower and BR007B was the latest. Three

varieties were purposely selected with different seed coat color to find if birds show differential preference for seed coat color. The varieties were: BR007B with red seeds; P9401 with white seeds (in place of SC283 used in 2015), and 1167048 — a brown seeded experimental hybrid with tannin (bird resistant) and referred to as Tannin line hereafter.

A detailed description of seven bag treatments is given in Table 1. Physical properties of synthetic fibers of the two nonwoven bags are given in Table 2. An important feature of the nonwoven materials of the synthetic bags is the mean pore size which was smaller than the size of sorghum pollen grain. The pollen of *Sorghum bicolor* series *sativa* and section *Eu-sorghum* on average measures 40  $\mu\text{m}$  (37-45) on the longer axis (Chaturvedi et al., 1991). Therefore, the new fabrics do not permit the entry of unwanted sorghum pollen grains and hence preserve the genetic identity of stocks. Duraweb® SG1 has higher thickness, tear strength and air permeability than duraweb® SG2 (Table 2).

Observations were made on all 5 panicles in each plot that were covered by a pollination bag type in a row of a variety whole-plot. Days to flowering was recorded for each row allocated to a bag type within the whole-plot of varieties. For each panicle in the study, data were collected on a scale of 1 to 5 to estimate the relative number of grains in the panicles after the bird damage, if any. Thus, the panicle scores for seed loss from bird damage corresponded to: 1 = 0%; 2 = 25%; 3 = 50%; 4 = 75% 5 = 100% damage. Among the grain-eating birds three species white-eyed parakeet, shiny cowbird and pigeons were most common and voracious (Figure 1). Quantitative data were collected on weight of five panicles (g). All five panicles of a treatment were threshed together in a head thresher and total seed weight was recorded in grams. A derived variable grain weight per panicle (g) was computed. Data were adjusted to five panicles per plot before computation since there were only four plants in treatments 6 and 7.

Analysis of seed health due to micro-environmental variation within bags was made by recording germination rate of seeds. Germination rate was measured as the per cent of germinated seeds in the laboratory under two conditions; normal and stress. The temperature in the normal condition was kept at 25°C and the substrate used for the test was Germitest Paper Roll on which 50 seeds were grown in two replications. A final germination count was taken after seven days following sowing. The stress environment simulated accelerated aging with stress under temperature of 42°C for 96 h. The substrate used for stress condition was Gerbox with screen and saturated saline solution. After the stress treatment, germination test was setup for the normal condition:

Treatment 1 (no bagging) was eliminated from germination test as no seed was available due to heavy bird damage. Treatment 2 (Kraft paper) also was affected by bird damage particularly for the white seeded and early flowering variety P9401 where all of the 8 observations in two environments and 4 replications had no seeds due to bird damage. Thus Kraft paper treatment was also eliminated from germination studies.

Data on occurrence of five pathogens (*Fusarium*, *Alternaria*, *Bipolaris*, *Phoma* and *Curvularia*) were collected by counting the number of infected grains from a sample of 50 grains. Any grain showing the signs of a pathogen was taken as diseased and counted so. Data were converted to percentages before analysis. The occurrence of pathogens was not exclusive since a seed could have been infected by multiple pathogens simultaneously.

Statistical analysis was performed using a split plot design following Sokal and Rohlf (2011). However, there was non-significant difference between error (a) and error (b) for all traits. The two errors were pooled to provide a more precise combined error variance by performing a factorial design analysis. Comparisons between means of treatments and interactions with varieties were made using least significant difference (LSD) at

**Table 1.** Description of pollination bag treatments.

Treatments	Treatment description
1	No bagging (control). Panicles were left uncovered by any bag
2	Kraft brown paper pollination bag normally used by sorghum breeders. The size can vary but 42 x 12 x 6 cm is commonly used made of Star paper of 60 g m <sup>-2</sup> mass
3	Kraft paper pollination bag covered with a plastic screen bag for extra protection following pollination and at seed formation
4	Used duraweb® SG1 pollination bag (see 6 below)
5	Used duraweb® SG2 pollination bag having smooth paper like surface (see 7 below)
6	New duraweb® SG1 pollination bag. It is a 3D bag of size 420 mm length x 140 mm width x 60 mm depth, made of layers of point-bonded nonwoven polypropylene with the goal of maximizing air permeability while also creating strength and the ability to block pollen. It has 60 g m <sup>-2</sup> mass
7	New duraweb® SG2 pollination bag having smooth paper like surface. It is a 3D bag of size 420 mm length x 140 mm width x 60 mm depth made from nonwoven polyester having 70 g m <sup>-2</sup> mass, thermally bonded, with a smooth paper-like surface similar to that of traditional duraweb®

**Table 2.** Specification of new nonwoven fabrics used in the manufacture of pollination bags (adapted from Scheffert et al., 2016).

Test	Units†	Duraweb® SG1	Duraweb® SG2
Polymers	-	Polypropylene	Polyester
Mass per unit area	g m <sup>-2</sup>	60	70
Thickness	mm	0.36	0.11
Tensile Strength (MD)	N/50mm	117	360
Tensile Strength (CD)	N/50mm	95	190
Tear Strength (MD)	N	37*	7.0
Tear Strength (CD)	N	46*	8.0
Mean Pore Size	µm	15	8.8
Air Permeability	l/m <sup>2</sup> /s	192	67

† MD: Machine directional, CD = Cross directional, N= Newton, L= litre, M= meter, S= second.

\* Test done using Trapezoidal test rather than the usual Trouser test used for SG2.



**Figure 1.** The most occurring birds on sorghum in the experiments were: 1. White eyed parakeet or parrot (*Psittacara leucophthalmus*); 2. The shiny cowbird or Chupim (*Molothrus bonariensis*); 3. Picazuro pigeon (*Patagioenas picazuro*).

5 and 1% probability. There was slight variation in the panicle number per treatment. Therefore, a covariance analysis, using panicle number as covariate, was performed for all traits with MINITAB 17 package. However, the covariance with panicle number

was not significant for any trait indicating no need for adjustment of treatment means for the effect of variable number of panicles. Therefore, the original analyses of variance without allowing for the regression of various traits on panicle number were used.

**Table 3.** Analysis of variance (mean squares) for quantitative traits recorded on three varieties and seven bag treatments.

Source	df	Panicle score	%SS for panicle score	Wt. of 5 panicles (g)	% SS Wt of 5 Panicles	Grain Wt. of 5 panicles (g)	% SS GW 5 panicles	Grain Wt. per panicle (g)	% SS GW per panicle
Reps	3	0.11	0.14	4183	1.12	1581	0.62	63.20	0.62
Variety, V	2	0.23	0.20	35139**	6.27	28385**	7.39	1135.4**	7.40
Bag type, B	6	34.46**	90.42	120537**	64.53	98708**	77.14	3948.3**	77.14
V x B	12	0.68**	3.59	5557	5.95	3385**	5.29	135.4**	5.29
Error	60	0.22	5.65	4134	22.13	1223	9.56	48.9	9.56

\*\* Significant at 1% level of probability, SS= Sum of squares.

## RESULTS

### Quantitative traits and bird damage

The analysis of variance showed that differences among bag types were highly significant ( $P < 0.01$ ) for all quantitative traits (Table 3). The varietal differences were also highly significant ( $P < 0.01$ ) for all traits except for panicle score (Table 3). Highly significant interactions of varieties x bags were observed for all traits except for weight of five panicles. Significant interaction for panicle score indicated differential response of varieties under different bags to bird attack which could have depressed the varietal differences to a non-significant level. However, the relative importance of bag types, varieties and interaction can be revealed by their contribution to the total sum of squares (SS). The bag types contributed the most to total SS for different traits (65 to 90%). Varieties contributed only 0.2 to 7.4% and interactions 4 to 6% for different traits. Thus interaction effects are not so important that variety specific bags are required. Mean values for main effects of varieties and bag types are given in Table 4 and Figures 2 and 3. In the presence of significant interactions mean values of main effects do not give precise comparison.

### Days to flowering

The analysis of variance (not given) for days to flowering of varieties showed highly significant differences among them with mean values of: variety 1167048 =  $71.25 \pm 0.30$ ; BR007B =  $73.50 \pm 0.30$  and P9401 =  $70.25 \pm 0.30$  days. Against LSD of 0.83 days at 5% probability both BR007B and 1167048 varieties were significantly later to flower than P9401. The variety BR007B was also significantly later flowering than 1167048 by 2.25 days. The earlier flowering white seeded variety P9401 was most vulnerable to bird damage as no seeds were left by birds under no bagging and Kraft paper treatment on this variety. The preference for this variety could also be a consequence of earlier grain availability for a longer period rather than just its low tannin content due to white grains.

### Panicle score (Bird damage)

Panicle score for overall variety means did not show large differences (Figure 2, Table 4). However, bag type treatment differences were significant and large between two groups of no bagging (Score 5 = 100% damage) and Kraft

paper (Score 4 = 75%) against a second group of all other treatments (3 to 7) that had almost no damage (Score 1.25, i.e 0 to 6%) and were non-significantly different (Figure 3, Table 4). All varieties were equally prone to bird damage under no bagging regardless of their seed coat colour. There was markedly more seed loss on white seeded variety P9401 compared with other two varieties under Kraft paper bags (Figure 4). Apparently, birds did prefer white followed by brown seeded variety when they had to search for seed under a bag (Figure 4). The bird damage, though small, was more on BR007B (red seeded) under treatments 3 than 7 (Figure 4). Mean values for panicle score indicate that treatments 1 (no bagging) and 2 (Kraft paper) were worse for panicle scores with 100 to 75% seed loss (Figure 4). Both of them were significantly inferior to all other treatments.

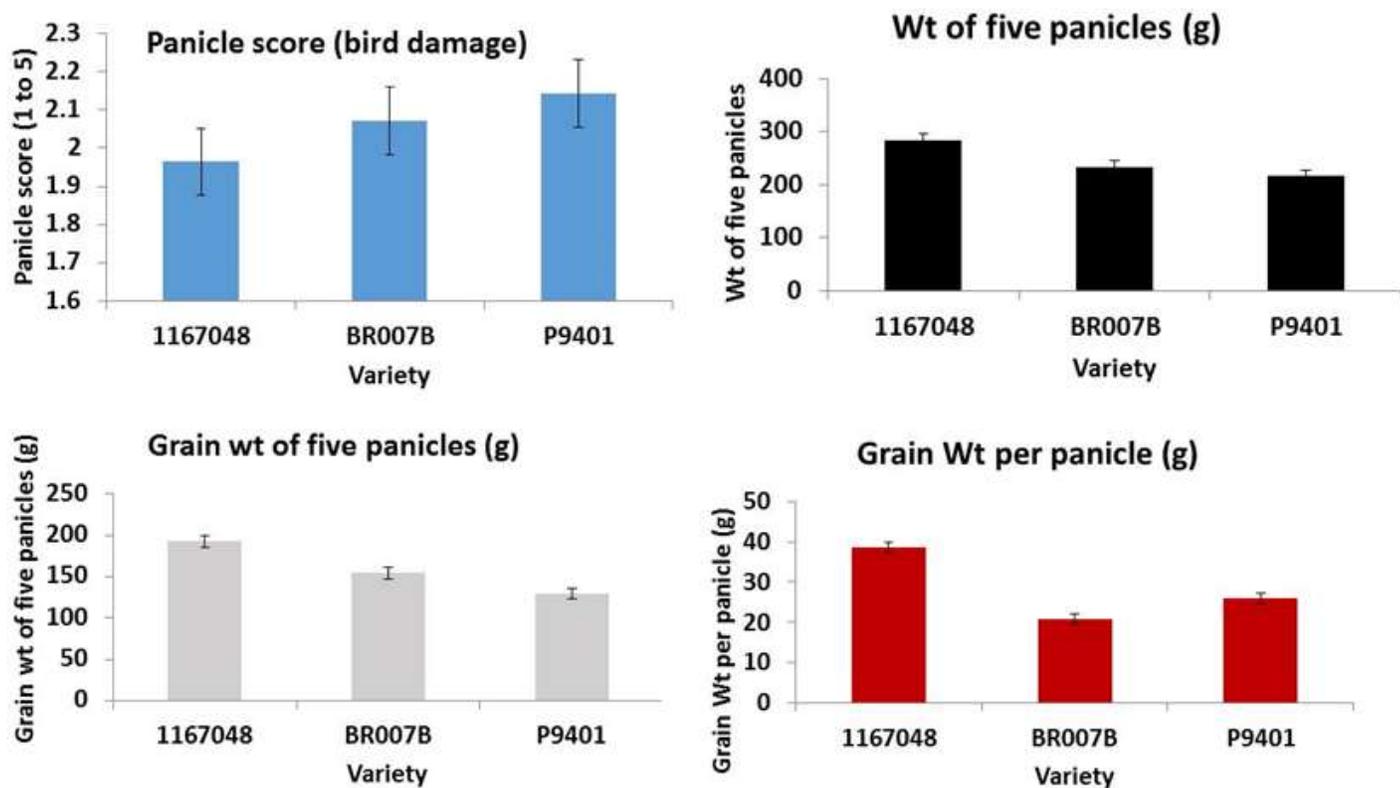
### Panicle weight

Interaction of varieties with bag types was non-significant for panicle weight (Table 3). Therefore, mean values of varieties and bag types can be compared. The hybrid 1167048 showed significantly higher panicle weight over P9401

**Table 4.** Mean values for quantitative traits recorded on three varieties and seven bag treatments.

Variety/treatment	Panicle score	Panicle Wt of 5 panicles (g)	Grain Wt of 5 panicles (g)	Grain Wt. per panicle (g)
<b>Varieties</b>				
1167048	1.96 <sup>A</sup>	284.06 <sup>A</sup>	192.40 <sup>A</sup>	38.48 <sup>A</sup>
BR007B	2.07 <sup>A</sup>	233.53 <sup>B</sup>	154.11 <sup>B</sup>	30.82 <sup>B</sup>
P9401	2.14 <sup>A</sup>	215.79 <sup>B</sup>	129.19 <sup>C</sup>	25.84 <sup>C</sup>
SE mean	0.09	12.15	6.61	1.32
LSD (5%)	0.25	34.37	18.70	3.73
Significance	NS	**	**	**
<b>Treatments</b>				
No bagging	5.00 <sup>A</sup>	60.63 <sup>C</sup>	18.35 <sup>B</sup>	3.67 <sup>B</sup>
Kraft Paper	4.00 <sup>B</sup>	145.21 <sup>B</sup>	34.57 <sup>B</sup>	6.92 <sup>B</sup>
Kraft + Plastic	1.25 <sup>C</sup>	303.99 <sup>A</sup>	200.47 <sup>A</sup>	40.09 <sup>A</sup>
Used duraweb® SG 1	1.00 <sup>C</sup>	304.72 <sup>A</sup>	208.80 <sup>A</sup>	41.76 <sup>A</sup>
Used duraweb® SG2	1.00 <sup>C</sup>	316.77 <sup>A</sup>	214.99 <sup>A</sup>	42.99 <sup>A</sup>
New duraweb® SG1	1.00 <sup>C</sup>	296.25 <sup>A</sup>	207.28 <sup>A</sup>	41.46 <sup>A</sup>
New duraweb® SG2	1.17 <sup>C</sup>	283.65 <sup>A</sup>	225.51 <sup>A</sup>	45.10 <sup>A</sup>
SE mean	0.13	18.56	10.10	2.02
LSD (5%)	0.37	52.50	28.57	5.71
Significance	**	**	**	**

NS= non-significant; \*\* Significant at 1% level of probability; Means that do not share same letter are significantly different at 5% level by Fisher's LSD method.

**Figure 2.** Bar diagrams of mean values ( $\pm$ SE) of varieties over all bag types for different traits.

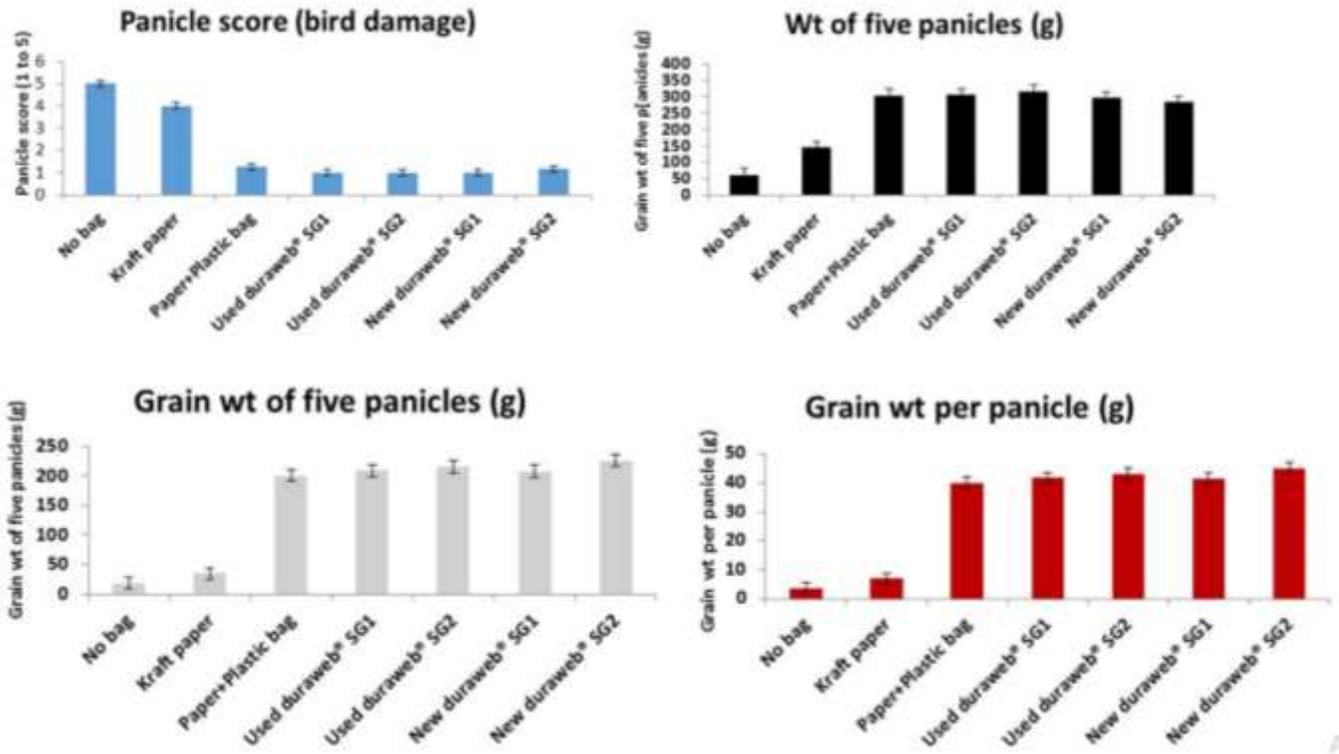


Figure 3. Bar diagrams for mean values (±SE) of bag treatments over all varieties for different traits.

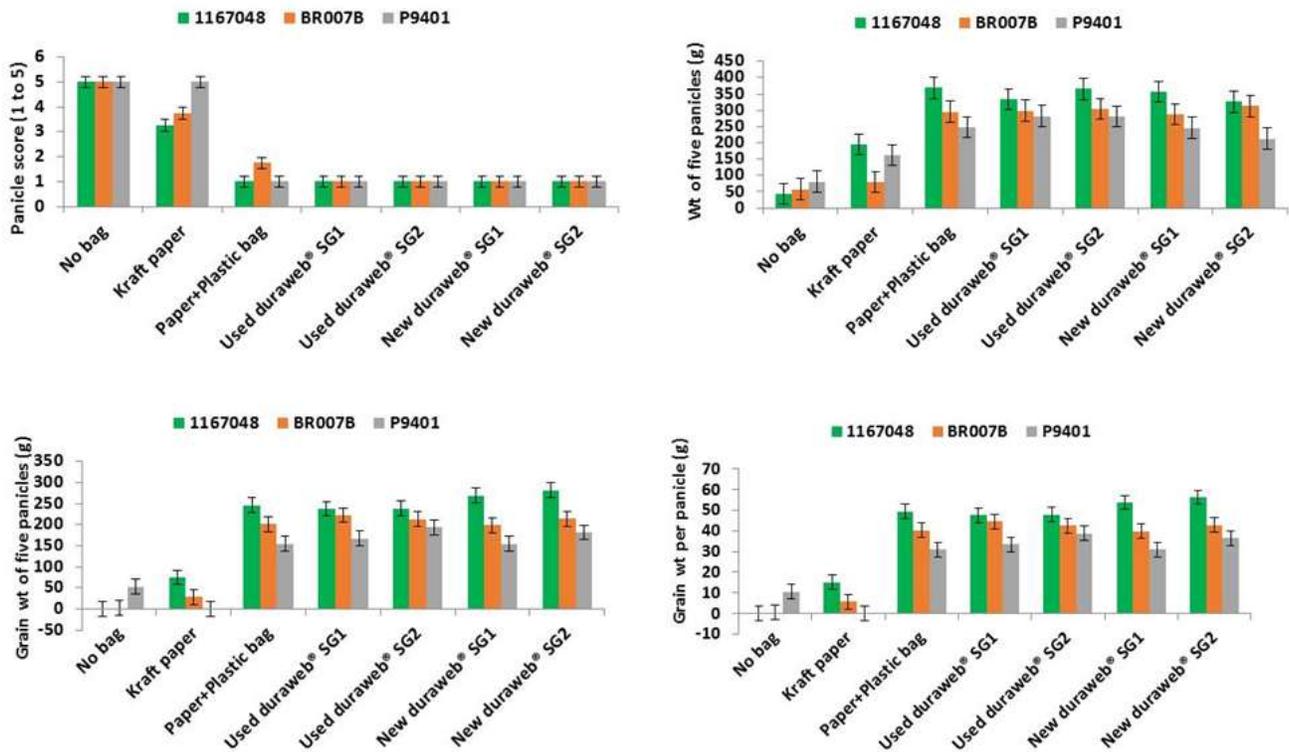


Figure 4. Interaction effects (±SE) of bag types x varieties for different traits.

**Table 5.** Mean germination per cent and standard errors for environments and varieties.

Environment/variety	Mean	SE
<b>Environment</b>		
Normal	90.41	0.92
Stress	78.22	0.92
Significance	**	
<b>Variety</b>		
1167048	86.96	1.09
BR007B	87.73	1.09
P9401	78.25	1.09
Significance	**	-

\*\* Significant at 1% level of probability.

(32%) and BR007B (22%). The varieties P9401 and BR007B did not differ significantly (Table 4). For bag treatments, no bagging was significantly the lowest. Kraft paper was significantly superior to no bagging but this treatment was significantly inferior to all other treatments from 3 to 7 which were all on par being statistically non-significantly different (Table 4 and Figure 3). Clearly covering of panicles even with a paper bag was better than no bagging at all.

### Grain weight

The hybrid 1167048 had a significantly higher grain weight than other two varieties, and in turn BR007B was superior to P9401 (Table 4, Figure 2). There was no difference between no bagging and Kraft paper treatments. These were, however, inferior to all other treatments from 3 to 7 that were on par for grain weight (Table 4, Figure 3). Interaction of varieties x treatments was primarily due to differences of no bagging and Kraft paper treatments over three varieties. No bagging produced more grain weight on P9401 and Kraft paper produced the lowest grain weight on this variety resulting in crossover interactions (Figure 4).

### Grain weight per panicle

Grain weight per panicle showed results similar to total grain weight for varieties, bag treatments and their interactions (Table 4 and Figures 2, 3, 4).

### Germination test

The analysis of variance for germination per cent showed significant differences between varieties and environments only. No significant differences were

detected between the bag treatments. Also none of the interactions such as variety x environment, bag treatment x environment and variety x treatment were significant (ANOVA not given). Therefore, mean values of varieties and environments can be compared without any complications.

The mean germination (%) in the normal condition was significantly higher (12% greater) than the stress condition (Table 5). Seeds of all varieties responded similarly to the stress condition. Overall, variety P9401 showed significantly lower mean germination (average 9% lower) than the other two varieties. The difference between the germination (%) of 1167048 and BR007B varieties was not significant. The lower germination of P9401 could be due to its differential storage response or physiological status of the seed at the harvest. The most important finding is the detrimental effect of stress (high temperature over consecutive four days) on seed germination highlighting the need for seed storage under ambient conditions.

### Disease pathogens

Interestingly, the different treatments did not vary significantly for the incidence of most pathogens except *Phoma* (Table 6). However, the mean occurrence of *Alternaria* was quite high in all bag treatments at 28 to 34% (Table 7) compared to the occurrence of *Fusarium*, *Bipolaris* and *Curvularia* under all bag types at less than 10% (Table 7). The differences among the varieties for all pathogens were significant showing that different varieties have variable susceptibility to mold pathogens (Table 6). White seeded variety P9401 showed higher occurrence of *Fusarium*, *Bipolaris* and *Curvularia* but lowest incidence of *Phoma*. Red seeded variety BR007B in general showed a lower disease occurrence than other varieties except for *Phoma* (Table 7). There were few significant differences between treatments (bag types, since there was no grain from Treatment 1) apart from the incidence of *Phoma*. In this regard (Table 7), Kraft paper (treatment 2) and Kraft paper plus plastic screen (treatment 3) were statistically on par with lowest incidence of *Phoma* (Table 7). New duraweb® SG1 and SG2 bags were on par and higher than but non-significantly different from Kraft and Kraft + screen treatments. However, the two used bags (treatments 4 and 5) had higher and comparable incidence of *Phoma*. Used duraweb® SG2 bag showed highest incidence of *Phoma* at 9%; significantly higher than the two new duraweb® bags (Table 7).

### Comparison of climate over 2015 and 2016

During the crop season (April to September), temperature showed a similar trend over two years with high correlations (Figure 5). There were three measurements available from daily temperature: high, average and low

**Table 6.** Analysis of variance (mean squares) for percent grains infected by five disease pathogens on three varieties following six bag treatments.

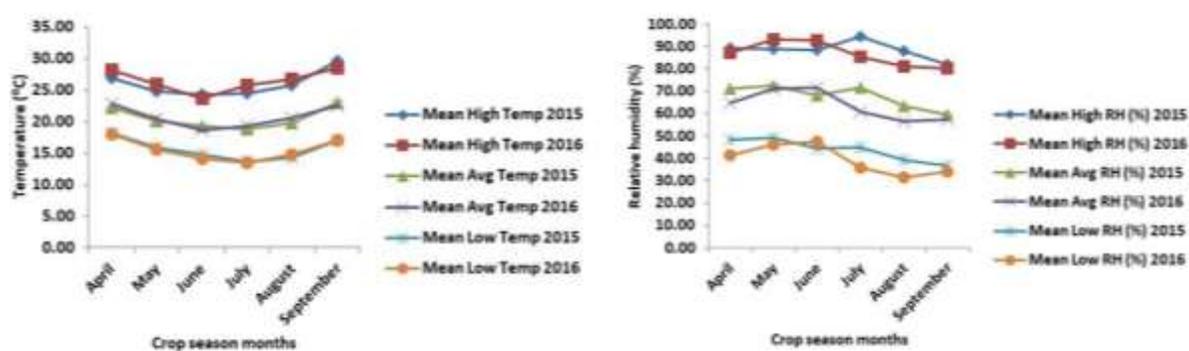
Source	df	Fusarium (% grains)	Alternaria (% grains)	Bipolaris (% grains)	Phoma (% grains)	Curvularia (% grains)
Reps	3	39.59	190.98	36.47	53.80	18.43
Variety, V	2	171.71*	1379.93**	213.93**	240.68**	36.77*
Bag type, B	5	41.79	77.53	36.41	85.00**	8.20
Error	57	44.85	86.92	16.69	22.45	10.69

\* Significant at 5% level of probability; \*\* Significant at 1% level of probability.

**Table 7.** Mean per cent ( $\pm$  SE) occurrence of different pathogens on grains (out of 50 grains) on three varieties and six bag treatments.

Variety/treatment	Fusarium	Alternaria	Bipolaris	Phoma	Curvularia
<b>Varieties</b>					
1167048	5.58 $\pm$ 1.37	39.75 $\pm$ 1.90	5.42 $\pm$ 0.83	5.00 $\pm$ 0.97	2.33 $\pm$ 0.67
BR007B	4.67 $\pm$ 1.37	25.42 $\pm$ 1.90	2.08 $\pm$ 0.83	7.67 $\pm$ 0.97	1.83 $\pm$ 0.67
P9401	10.13 $\pm$ 1.56	27.88 $\pm$ 2.17	8.45 $\pm$ 0.95	0.88 $\pm$ 1.10	4.38 $\pm$ 0.76
Significance	*	**	**	**	*
<b>Treatments†</b>					
Kraft Paper	8.42 $\pm$ 2.45	28.43 $\pm$ 3.40	3.57 $\pm$ 1.49	0.93 $\pm$ 1.73	2.77 $\pm$ 1.19
Kraft + Plastic	5.67 $\pm$ 1.93	30.50 $\pm$ 2.69	4.50 $\pm$ 1.18	2.50 $\pm$ 1.37	2.00 $\pm$ 0.94
Used duraweb® SG1	9.16 $\pm$ 1.93	34.00 $\pm$ 2.69	4.17 $\pm$ 1.18	6.67 $\pm$ 1.37	2.83 $\pm$ 0.94
Used duraweb® SG2	7.67 $\pm$ 1.93	31.33 $\pm$ 2.69	5.33 $\pm$ 1.18	8.83 $\pm$ 1.37	3.00 $\pm$ 0.94
New duraweb® SG1	5.67 $\pm$ 1.93	34.00 $\pm$ 2.69	8.67 $\pm$ 1.18	4.00 $\pm$ 1.37	2.16 $\pm$ 0.94
New duraweb® SG2	4.17 $\pm$ 1.93	27.83 $\pm$ 2.69	5.67 $\pm$ 1.18	4.17 $\pm$ 1.37	4.33 $\pm$ 0.94
Significance	NS	NS	NS	**	NS

† No bagging treatment is excluded since no seed could be saved from birds; \* Significant at 5% level of probability; \*\* Significant at 1% level of probability; NS= Non-significant.

**Figure 5.** Comparison of mean monthly temperature and relative humidity (%) over 2015 and 2016 during the sorghum crop season (April to September). Left: monthly mean of daily high, low and average temperature ( $^{\circ}$ C); Right: monthly mean of daily high, low and average relative humidity (%).

temperature. Mean of these measurements were taken for each month. Similar data were available for relative humidity (%). Correlations for temperature were significant between years; mean high temperature ( $r = 0.85$ ;  $P < 0.05$ ), mean average temperature ( $r = 0.94$ ;

$P < 0.01$ ) and mean low temperature ( $r = 0.98$ ;  $P < 0.01$ ). Similar trends for temperature were observed for the whole years' data. Relative humidity (%) showed non-significant correlations for all three humidity measurement ( $r$  for mean low = 0.73;  $r$  for mean

average = 0.66;  $r$  for mean high = 0.40). Figure 5 shows that there was lower relative humidity during July and August in 2016 than in 2015. There was also a non-significant relationship for wind velocity between the two years during the crop season ( $r$  for mean low = 0.75;  $r$  for mean average = 0.49). This means whatever differences were observed between 2015 and 2016 were determined by the differences in humidity and wind speed.

## DISCUSSION

Sorghum breeders use Kraft paper pollination bags for selfing, crossing, generation advance of selected lines, maintenance of germplasm accessions and for protecting against birds in isolation plots of small sizes or nurseries grown in the off-season with little alternative food sources for birds (Ormerod and Watkinson, 2000; Gitz et al., 2013, 2015).

Dahlberg et al. (2011) reported that about 40,000 germplasm lines are maintained in the US sorghum collection alone besides almost every sorghum-growing country having its own germplasm collections. Maintenance of these accessions and numerous lines in the breeding nurseries all over the world need protecting from contamination with foreign pollen through the use of pollination bags.

The traditional paper bags offer weak protection and are easily torn open in the rainy season with high winds and severe bird pressure. However, the recent studies have shown that alternatives to paper pollination bags provided by nonwoven synthetic materials are stronger, offering almost perfect protection against being torn off by birds in search of food and/or from high winds and rains. Research shows they also provide better micro-climatic environment for healthy seed development (Gitz et al., 2013; 2015; Schaffert et al., 2016; Gaddameedi et al., 2017). The new nonwoven duraweb® materials are specifically designed to be used as pollination bags for various crops with porosity smaller than the pollen size to avoid contamination but porous enough to allow air flow for maintaining ambient humidity and temperature within them (Adhikari et al., 2014; Bonneau et al., 2017; Hayes and Virk 2016; PBS International, 2016).

The statistical analysis performed in this paper considered two aspects; the effect of variable plant stands and design of the experiment. Sorghum being cultivated in dry and rainfed conditions often has differential plant stand resulting from uneven germination and seedling survival due to soil and climatic conditions or attack by insects. In such situations, adjustment of means for the differential plant stand would be required which is conveniently performed by analysis of covariance that combines the features of analysis of variance and regression (Sokal and Rohlf, 2011). This analysis was performed but no trait was found to be significantly influenced by the variable plant stand. Thus

adjustments of means for their covariance with plant stand were not justified in the present case. Secondly, the experiment was laid out in a split plot design but the analysis was performed as a factorial design because error (a) for whole plots and error (b) for sub-plots were non-significantly different and pooling them together in a factorial design was justified to provide a precise estimate of error variance with more degrees of freedom.

The present results are in complete agreement with those obtained in 2015 (Schaffert et al., 2016). In general, over both years Tannin hybrid (1167048) was highest scorer for all traits followed by BR007B and white seeded variety SC283 or P9401. The bag type treatments fell in two clear groups. The first group was of no bagging and Kraft paper, scoring the lowest for all traits. The second group was of Kraft paper + plastic screen as well as all nonwoven bags, which scored the highest for all traits. This conclusion is supported by the high correlation of temperature during the crop season over the two years. Similar but non-significant trend existed for relative humidity and wind velocity.

Bird damage in 2016 was higher than in 2015 at Sete Lagoas (Brazil). Therefore, all varieties irrespective of their seed coat colour were equally prone to bird attack. In 2015, bird damage under no bagging and paper bag treatment was high on white and red seeded varieties compared with no bird damage on the brown seeded hybrid with tannin (Schaffert et al., 2016). Thus when there is choice, birds preferred white seeded variety P9401 or SC283 more than others.

Tannin is a polyphenolic biomolecule that binds to proteins and various other organic compounds including amino acids and alkaloids. The tannins produce astringency that is known to cause the dry and 'pucker' feeling in the mouth of birds following the consumption of unripe seed (McGee, 2004). Therefore, birds avoid seeds with tannin in the presence of alternatives. Katie and Thorington (2006) reported that tannin compounds are found in many species of plants and are known to provide protection against predation (birds). The presence of tannins deters birds unless there is no other nearby food source available. The mean bird damage on varieties in 2016 was in the order 1167048 < BR007B < P9401 and was similar to that observed in 2015 though the intensity was higher.

The results of 2016 confirm that no bagging and Kraft paper bags offered the least protection, with damage of 100 and 75%. When the pressure is high, as in 2016, the paper bags are almost fully torn open by birds and the plastic screen bags can even be removed by birds during multiple visits in search of food within them. No seed recovery under Kraft bags on white-seeded variety P9401 in 2016 indicated a high bird pressure in 2016 and that birds preferred white seeds over other colours. Compared with 2016, the bird pressure during 2015 winter season was medium as there were alternative food sources due to above average rainfall. Unlike 2016 no

bird damage was observed on the tannin variety and the birds preferred white and red seeded varieties. Compared with 100 and 75% seed loss under no bagging and Kraft paper bags in 2016 the estimated seed loss from uncovered panicles in 2015 was about 50% and that from those covered by Kraft paper bags was about 20 to 25%. This means the bird pressure in 2015 was about the half of 2016.

However, all bag types other than paper bags including the new and used nonwoven bags provided a strong protection against birds with nearly no damage to grains (1 to 1.25 score in 2016) in both years. Thus the new nonwoven materials have strength equal to Kraft paper bags plus protective plastic screen, although the latter requires a second visit to apply adding labour cost, compared to a single visit for the former.

The analysis of variance (Table 3) showed a significant variety x bag type interaction for panicle score, grain weight of five panicles and grain weight per panicle. However, the interaction was not significant for the total weight of five panicles. Are these significant interactions really suggesting that variety specific pollination bags be used? This can be investigated by delineating the per cent contribution of each item in the analysis of variance to the total sum of squares (SS). Interestingly, the contribution of interaction SS to the total SS for all traits is very small varying from 4 to 6% only (Table 3).

Similarly, the varietal contribution is also small being only 0.2 to 7.4%. On the other hand, the bag types accounted for 65 to 90% of the total SS for various traits. This clearly brings out the importance of bag type and perhaps the selection of appropriate bag type would exclude the need of choosing the variety specific bags in view of little contributions of interactions to the total SS despite being significant.

Fungi belonging to more than 40 genera are reported to be associated with sorghum grain mold (Thakur et al., 2006). Of the various fungal species that cause grain mold in sorghum the most important are: *Fusarium* spp., *Curvularia lunata*, *Alternaria alternata*, *Phoma sorghina*, *Bipolaris australiensis* (Navi et al., 2005; Thakur et al., 2006). The occurrence of these fungi on grains was studied in the present investigation. The three varieties significantly differed for the occurrence of various pathogens showing their differential susceptibility to these grain mold pathogens, but the pollination bags treatments did not differ significantly for four of the pathogens. The only observable significant difference between bag types was for the *Phoma* pathogen. The used duraweb® SG1 and SG2 bags showed significantly higher *Phoma* attack than all other bag types including the new duraweb® SG1 and SG2 (Table 7). The used duraweb® SG2 showed the highest incidence of 9%.

This experiment did not test whether any of the pathogens survived in the used duraweb® bags the possibility of survival of *Phoma* cannot be ruled out. The best practice would thus demand treating the used

bags with fungicides or washing them clean with soft detergent before applying on inflorescences for pollination purposes. Alternatively, autoclaving the bags may preclude the possibility of survival of mold pathogens (Hayes and Virk, 2016).

A preliminary economic analysis was performed by Schaffert et al. (2016) for a sorghum breeding programme rather than a commercial seed production situation. It was pointed out that small quantities of seed are produced for several lines or plants in the segregating generations. Pollination bags do not just avoid contamination but also protect against birds, since loss of any progeny is a permanent loss for the breeding programme. We have seen in years like 2016 the loss from bird attack can be severe. There was 100% seed loss with no bagging and 75% with Kraft paper bags but the new nonwoven bags (used or new) showed no seed loss from birds. On average new and used duraweb® bags resulted in heavier weight of five panicles (195 to 218% greater), more total grain weight of five panicles (600 to 652% more) and higher average grain weight per panicle (599 to 652% greater) compared to the Kraft paper treatment (Table 4). This is a significant economic benefit from the novel bags under high bird pressure and confirms the results of Schaffert et al. (2016) under medium bird pressure. The greater strength of the novel bags reduces the number of plants required to produce a target seed yield, as a surplus to allow for bird damage is not necessary. In addition to avoiding sowing extra seeds in compensation for bird loss, extra labour to patrol the fields to replace damaged bags as and when required can be eliminated.

This study confirms the observation of Hayes and Virk (2016) that duraweb® bags are re-usable but it is still a preliminary study. Experiments testing how many times a duraweb® bag can be used need to be planned with different cleaning treatments such as washing with detergent, sun-drying and autoclaving to observe persistence of diseases. If these bags can be used multiple times then the actual cost of bags is reduced by the times the bag is reused and hence making them more economical than when the initial higher investment is considered.

These results, however, confirm results of previous experiments and suggest that while pollination bags made of novel nonwoven fabrics are superior there still is a need to explore economic implications more fully, and to compare the seed harvest of different bags to the micro-environmental differences within them.

## Conclusion

Experiments over two years revealed the superiority of nonwoven pollination bags over the Kraft paper bags for sorghum breeding where mold or birds are problems. These bags virtually eliminated bird damage and resulted

in higher total panicle weight, total grain weight and average seed weight per panicle across three varieties of sorghum. The work also provided the evidence that novel pollination bags can be re-used provided they are cleaned, sterilized or chemically treated between seasons. Consequent upon results it is recommended that sorghum breeders may replace paper bags with those made from nonwoven synthetic materials.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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