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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 μm (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 ⁻² 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 ⁻² 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 ⁻² 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 ⁻²	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 ² /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 ± 0.30 ; BR007B = 73.50 ± 0.30 and P9401 = 70.25 ± 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.

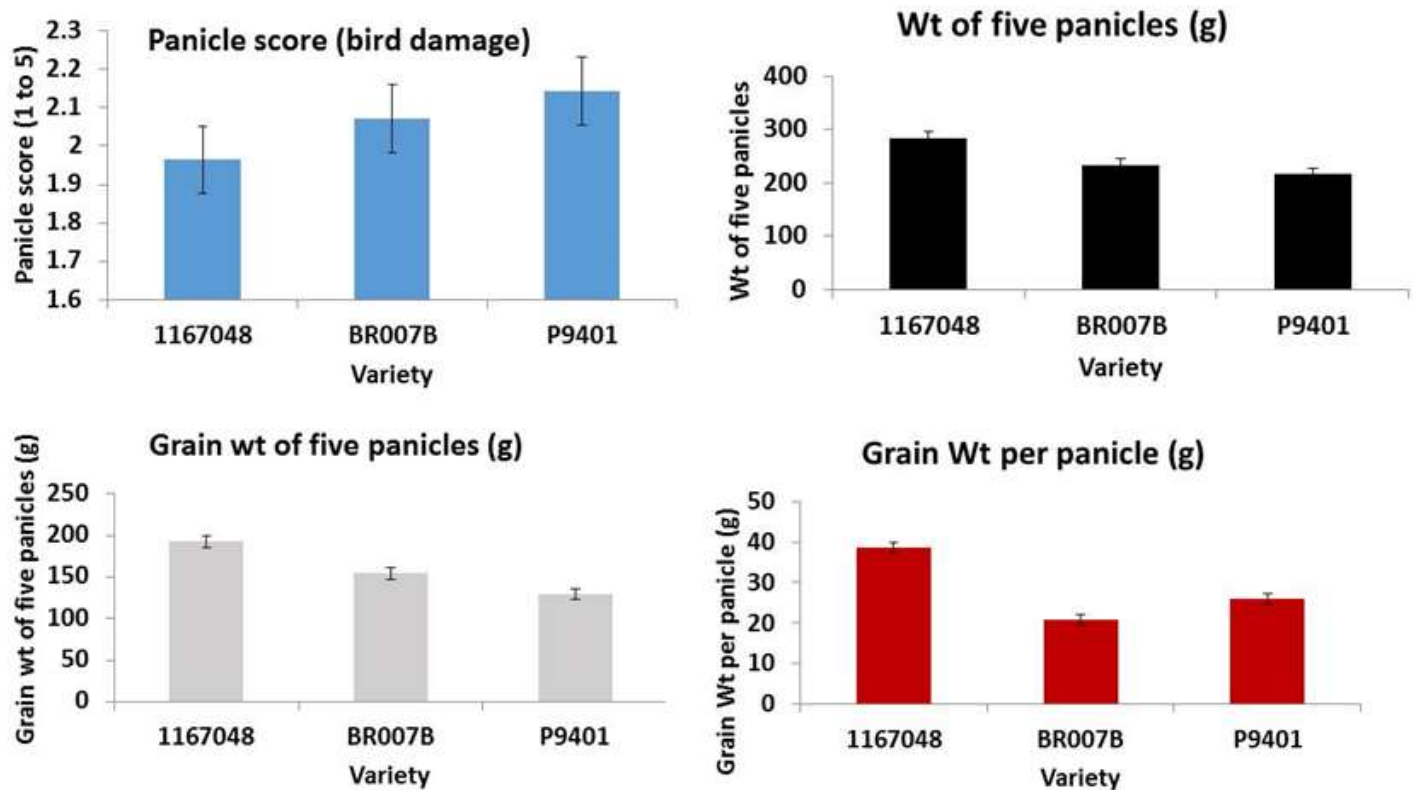
Panicle weight

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)
Varieties				
1167048	1.96 ^A	284.06 ^A	192.40 ^A	38.48 ^A
BR007B	2.07 ^A	233.53 ^B	154.11 ^B	30.82 ^B
P9401	2.14 ^A	215.79 ^B	129.19 ^C	25.84 ^C
SE mean	0.09	12.15	6.61	1.32
LSD (5%)	0.25	34.37	18.70	3.73
Significance	NS	**	**	**
Treatments				
No bagging	5.00 ^A	60.63 ^C	18.35 ^B	3.67 ^B
Kraft Paper	4.00 ^B	145.21 ^B	34.57 ^B	6.92 ^B
Kraft + Plastic	1.25 ^C	303.99 ^A	200.47 ^A	40.09 ^A
Used duraweb® SG 1	1.00 ^C	304.72 ^A	208.80 ^A	41.76 ^A
Used duraweb® SG2	1.00 ^C	316.77 ^A	214.99 ^A	42.99 ^A
New duraweb® SG1	1.00 ^C	296.25 ^A	207.28 ^A	41.46 ^A
New duraweb® SG2	1.17 ^C	283.65 ^A	225.51 ^A	45.10 ^A
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 (±SE) of varieties over all bag types for different traits.

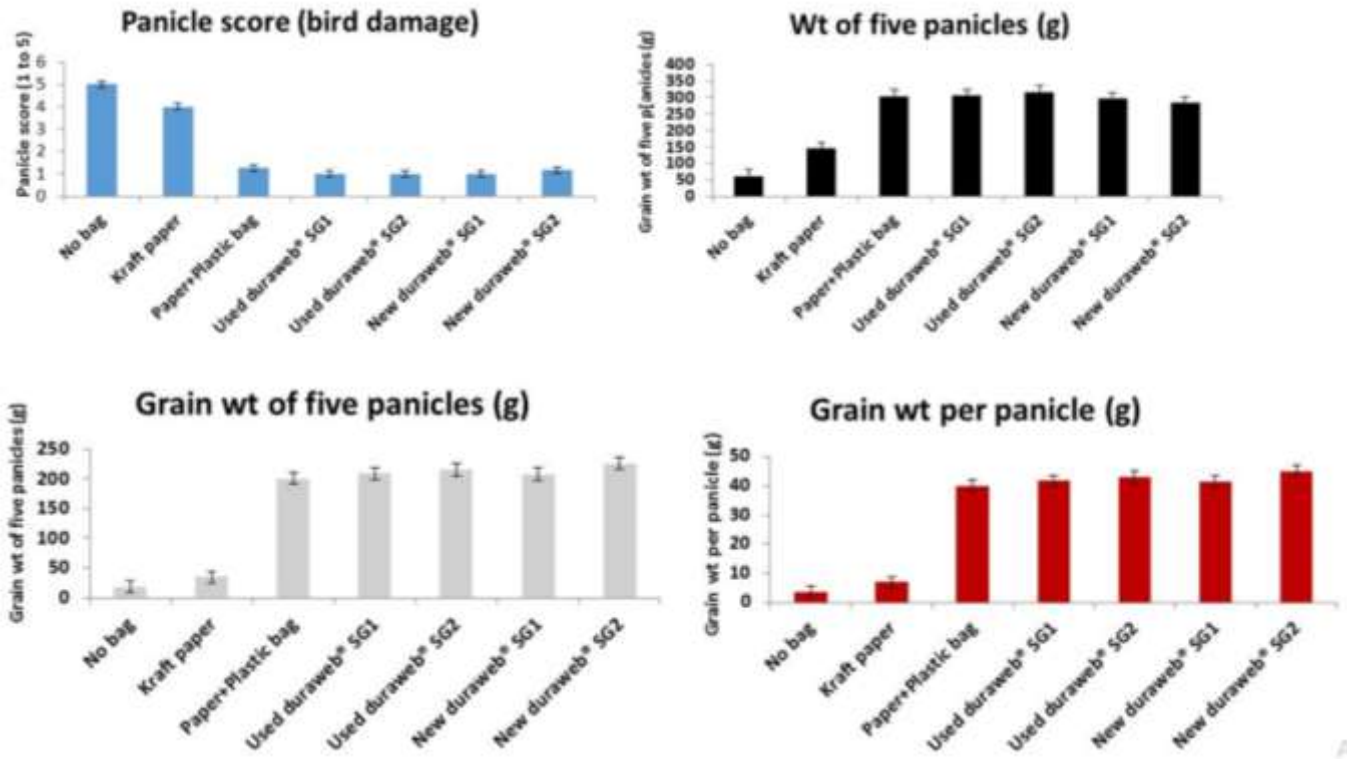


Figure 3. Bar diagrams for mean values (±SE) of bag treatments over all varieties for different traits.

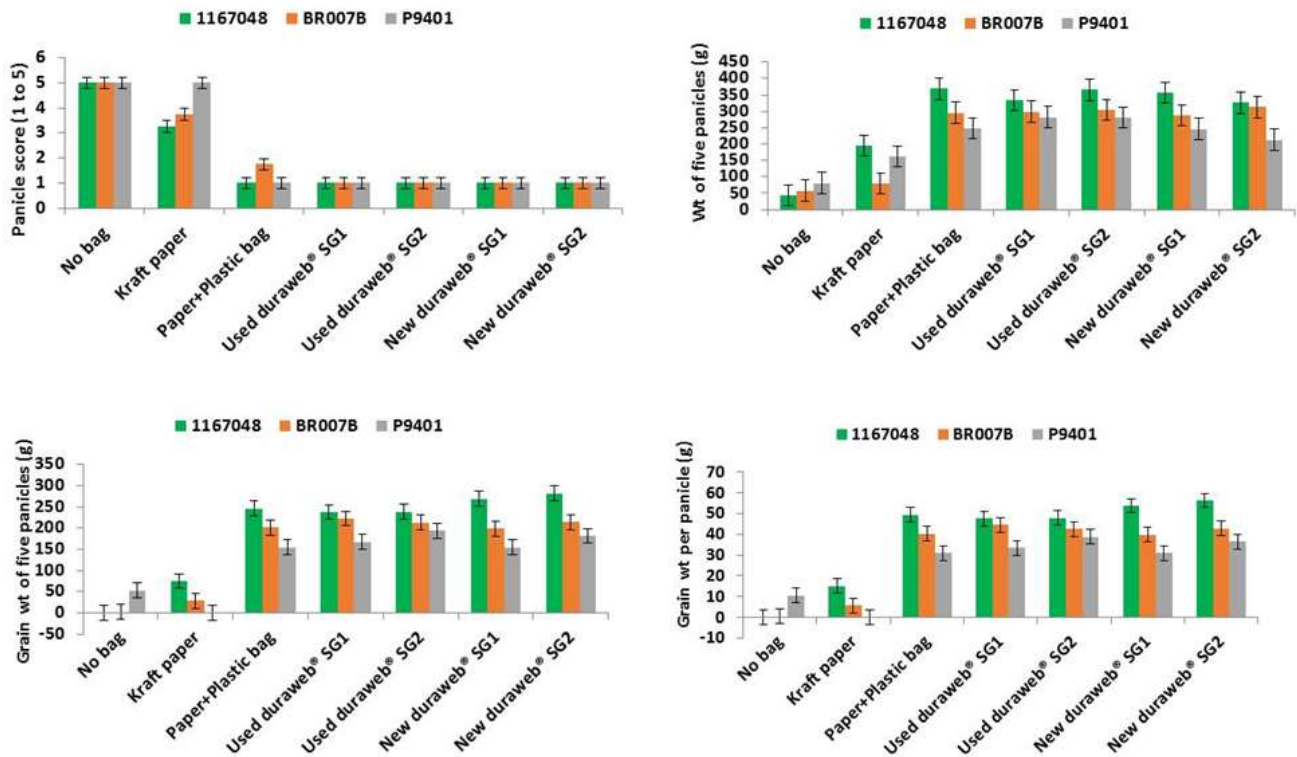


Figure 4. Interaction effects (±SE) of bag types x varieties for different traits.

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

Environment/variety	Mean	SE
Environment		
Normal	90.41	0.92
Stress	78.22	0.92
Significance	**	
Variety		
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.

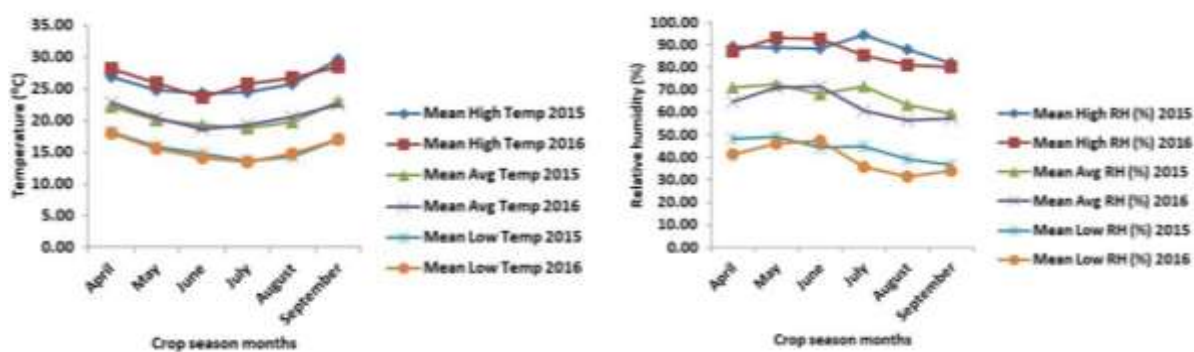
Source	df	Fusarium (% grains)	Alternaria (% grains)	Bipolaris (% grains)	Phoma (% grains)	Curvularia (% grains)
Reps	3	39.59	190.98	36.47	53.80	18.43
Variety, V	2	171.71*	1379.93**	213.93**	240.68**	36.77*
Bag type, B	5	41.79	77.53	36.41	85.00**	8.20
Error	57	44.85	86.92	16.69	22.45	10.69

* 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
Varieties					
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	*	**	**	**	*
Treatments†					
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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Full Length Research Paper

Field assessment of disease resistance status of some newly-developed early and extra-early maize varieties under humid rainforest conditions of Nigeria

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Periodic assessment of resistant status of genetic materials in a breeding program is an important activity to ensure its continued progress. Forty newly-developed early and extra-early maize varieties were evaluated under natural field infection conditions for two years to assess their resistance status to some common diseases prevalent in the humid rainforest agro-ecology, and to determine effect of the diseases on grain yield and other agronomic characters. The experiment was laid out using a 5 x 8 alpha lattice design with three replications. Data were recorded on flowering traits, disease scores as well as yield and yield components. Data collected were subjected to analysis of variance, correlation and regression analyses. Results revealed that the varieties were significantly different for flowering traits, as well as yield and yield components except ears per plant, ear aspect and plant aspect. For disease scores, the varieties were not significantly different except for *Helminthosporium maydis*. There was a differential response of the early and extra-early maize varieties under the field evaluation conditions. However, all varieties maintained their resistance level against streak, northern leaf blight, southern leaf blight and smut. Although, none of these diseases significantly reduced yield, scores for *Curvularia* leaf spot and rust disease significantly exceeded the resistance threshold, suggesting an urgent attention is needed for the management of the diseases before the damages reach economic threshold.

Key words: Blight, *Curvularia*, maize, rainforest, streak.

INTRODUCTION

Maize (*Zea mays* L.) is an important staple cereal in sub-Saharan Africa because of its great economic value and wide adaptation to all agro-ecological zones in the region. It plays a critical nutritional role in human and animal diet. However, maize production in tropical Africa is constrained by a number of stress factors which could be biotic and abiotic. Important biotic stress in maize

production is a complex of pests and diseases that significantly reduce the quantity and quality of production. Grain yield losses ranging from 1 to 70% have been reported due to some of the major diseases, which depend on factors such as genetic constitution of the cultivars, stage of growth at the time of infection, and environmental conditions (Bua and Chelimo, 2010).

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The maize plant is susceptible to many diseases that affect yield and quality of the crop. These diseases are caused by both infectious and non-infectious causal agents. Infectious causal agents are biological organisms that increase their population on diseased plants and then are spread to healthy plants, causing disease. They include fungi, bacteria, viruses, nematodes, and other organisms that are commonly thought of as plant pathogens. The losses due to diseases cannot be adequately estimated because disease symptoms are found on virtually all maize plants, and it is rather very difficult, if not impossible to create conditions where the plant is completely free from disease. The greatest losses caused by disease are probably from those diseases that occur annually.

Among the diseases of economic importance in maize production in the humid tropics of Nigeria is streak. The disease is caused by a geminivirus that is transmitted by viruliferous leafhoppers of the genus *Cicadulina mbila*. Incidence of maize streak is estimated at 60% across all African agro ecosystems where maize is grown (De Vries and Toenniessen, 2001) and it is considered as the most widespread biotic constraint to maize production. Rusts is another important maize disease caused by a fungus (*Puccinia polysora*). The pathogen has distinctive reproductive structures called pustules that erupt through the surface of leaves, stalks, or husks and produce spores called urediniospores which are round and red-brick in colour scattered on the leaf surface and occur on both leaf surfaces. Severe infections can lead to defoliation and premature senescence (CIMMYT, 2004).

Northern corn leaf blight (NCLB) is caused by a fungus *Helminthosporium turcicum*. Its symptom is typified by long (length: 2 to 15 cm) lesions with tapered ends that is gray-green to tan lesions in colour on lower leaves at the beginning, but can spread to all leaves and husks with secondary infections. The disease is prevalent in areas of high altitude and cold regions but its incidence has been noticed among some inbred lines in the humid rainforest locations in Nigeria lately. Southern corn leaf blight is another disease of notable economic importance caused by a fungus *Helminthosporium maydis*. It is favoured by warm temperature, high rainfall and high humidity. Typically, it is more of a problem in the south-western region of Nigeria than northern corn leaf blight (CIMMYT, 2004). Other important diseases of maize in this region are *Curvularia* leaf spot (CLS): caused by the fungus *Curvularia lunata* (Wakker) Boedijn which results in yield losses up to 20 to 30% (Dai et al., 1996; Lui et al., 1997) and corn smut caused by *Ustilago maydis*.

Southwestern zone of Nigeria is characterized by high temperature, rainfall, and relative humidity, conditions, which favour high disease incidence and build-up. It is therefore a hotspot for testing resistance status of newly developed maize varieties and hybrids in the sub-region.

Although, the incidence and severity of most of these diseases can be reduced by chemical control methods

ranging from seed dressing to foliar spraying, host plant resistance provides the most economical management option to farmers, which is also environmentally friendly. The scientists at the International Institute of Tropical Agriculture (IITA) and national agricultural research stations in Nigeria had, in time past, worked hard to develop maize germplasm sources that are resistant to most maize diseases of economic importance in the region and routinely generate new maize genetic materials from these germplasm sources so that, resistance to those common diseases are automatically acquired by the new materials.

However, most times, resistance breakdown due to segregation of genes for resistance, mutation of the pathogens or introduction of new morphotypes or ecotypes of the pathogens cause disease. Therefore, it is important to periodically examine the level of resistance of the newly developed maize genetic materials to these common diseases. This could be carried out in a greenhouse facility where, the inoculum of the diseases is artificially applied and the symptoms recorded. Another alternative is the use of natural field screening at hot spot where such disease is endemic.

The objectives of the study were to (i) assess resistance status of early and extra-early maize varieties to some common disease conditions, prevalent in the humid rainforest agro-ecology, and (ii) determine effect of the diseases on grain yield and other agronomic characters of the varieties.

MATERIALS AND METHODS

Location

The study was carried out at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife (7°28'N, 4°33'E, rainfall 1150 mm, altitude 224 m above sea level) which is located in the humid rainforest ecology of southwestern Nigeria. The experiment was conducted during the cropping seasons of 2014 and 2015, when disease incidence is usual maximum.

Plant materials and field layout

Forty early and extra-early maize varieties with divergent reactions to biotic and abiotic stresses developed for the mid-altitude and sub-humid agro-ecologies of west and central Africa by the Maize Improvement Unit of the (IITA) were used for this study. Brief description of the characteristics of 40 maize varieties was given in Table 1. The experimental field had been left to fallow for a year. The land was ploughed twice, and harrowed two weeks before the layout and planting was done. A 5 x 8 alpha lattice design with four replications was used for the evaluation of the genetic materials. Each plot consisted of a two-row, 5 m long, spaced 0.75 m apart with, within row spacing of 0.5 m.

The planting was done manually on the 25th July, 2014 and 13th June, 2015. Three seeds were sown per hill. Atrazine was sprayed as a pre-emergence herbicide, immediately after planting at the rate of 1.5 litres per ha. Two weeks after planting, the three seedlings per stand were thinned to two to maintain plant population of 66,666 plants per hectare. Three days later, a compound fertilizer,

Table 1. Description of the genetic materials evaluated at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife. Nigeria and their reactions under stress.

S/N	Pedigree	Maturity	Endosperm colour	Endosperm type	Reaction to drought	Reaction to <i>Striga</i> infestation
1	TZEE-W STR BC ₅	Extra-early	White	Normal	Susceptible	Highly resistant
2	TZE-WPOP DT STR C ₄	Early	White	Normal	Resistant	Resistant
3	2009 TZE-WDT STR	Early	White	Normal	Resistant	Resistant
4	2000 SYN EE-W STR	Extra-early	White	Normal	Susceptible	Resistant
5	EVDT-Y 2000 STR	Early	Yellow	Normal	Susceptible	Resistant
6	DTE STR-Y SYN 2000 POP C ₂	Early	Yellow	Normal	Resistant	Resistant
7	2008 DTMA-Y STR	Early	Yellow	Normal	Resistant	Resistant
8	2009 DTE-Y STR	Early	Yellow	Normal	Resistant	Resistant
9	EVDT-Y 2000 STR QPM	Early	Yellow	QPM	Susceptible	Resistant
10	DTE-W STR SYN	Early	White	Normal	Resistant	Resistant
11	2008 TZEE-Y STR	Extra-early	Yellow	Normal	Susceptible	Resistant
12	2009 TZEE-OR ₂ STR QPM	Extra-early	Orange	QPM	Susceptible	Resistant
13	2009 TZEE-OR ₁ STR	Extra-early	Orange	Normal	Susceptible	Resistant
14	2000 SYN EE-W STR QPM	Extra-early	White	QPM	Susceptible	Resistant
15	99 TZEE-Y STR QPM	Extra-early	Yellow	QPM	Susceptible	Resistant
16	2008 SYN EE-W DT STR	Extra-early	White	Normal	Resistant	Resistant
17	TZEE-W POP STR C ₅	Extra-early	White	Normal	Susceptible	Resistant
18	TZEE-WPOP STR 104 BC ₂	Extra-early	White	Normal	Susceptible	Resistant
19	DTSTR-Y SYN POP C ₃ F ₁	Early	Yellow	Normal	Resistant	Resistant
20	SYN DTE STR-Y	Early	Yellow	Normal	Resistant	Resistant
21	EVDT-Y 2000 STR QPM	Early	Yellow	QPM	Resistant	Resistant
22	2011 TZE-Y DT STR	Early	Yellow	Normal	Resistant	Resistant
23	TZE-Y POP DT STR QPM	Early	Yellow	QPM	Resistant	Resistant
24	EVDT-W 2008 STR	Early	White	Normal	Resistant	Resistant
25	2009 TZEE-OR ₁ DT STR QPM	Extra-early	Orange	QPM	Resistant	Resistant
26	2004 TZEE-W POP STR C ₄	Extra-early	White	Normal	Resistant	Resistant
27	SYN DTE STR-W	Early	White	Normal	Resistant	Resistant
28	DT-W STR SYN	Early	White	Normal	Resistant	Resistant
29	2011 TZE-W DT STR SYN	Early	White	Normal	Resistant	Resistant
30	2008 DTMA-Y STR	Early	Yellow	Normal	Resistant	Resistant
31	EV DT-Y 2008 STR	Early	Yellow	Normal	Resistant	Resistant
32	DTE STR-W SYN POP C ₃ F ₁	Early	White	Normal	Resistant	Resistant
33	2004 TZEE-YPOP STR C ₄	Extra-early	Yellow	Normal	Resistant	Resistant
34	2013 DTE STR-W SYN F ₁	Early	White	Normal	Resistant	Resistant

Table 1. Contd.

35	2011 DTE Y STR SYN	Early	Yellow	Normal	Resistant	Resistant
36	2013 DTE STR-Y SYN F ₁	Early	Yellow	Normal	Resistant	Resistant
37	2012 TZE-W POP DT C ₄ STR C ₅	Early	White	Normal	Resistant	Resistant
38	TZEE-Y POP STR C ₂	Extra-early	Yellow	Normal	Moderately resistant	Moderately resistant
39	TZEE-W POP STR QPM C ₂	Extra-early	White	QPM	Moderately resistant	Moderately resistant
40	TZEE-Y POP STR C ₂ QPM	Extra-early	Yellow	QPM	Moderately resistant	Moderately resistant

NPK 15-15-15, was applied by side placement method at the rate of 60 kg per ha and 5 weeks after planting, additional 30 kg N per ha was applied as top dressing using urea fertilizer. Weed control at this stage was carried out by hand weeding. No disease control measure was applied throughout the period of the experiment except seed dressing with Apron-plus to prevent rodents and birds from picking the seeds before and during germination.

Data collection

Data were recorded on emergence percentage, number of days to 50% silking and 50% anthesis and anthesis-silking interval was calculated as the difference between the days to silking and anthesis. Plant height was recorded as the average heights of 10 plants per plot from the soil level to the first tassel branch. The mean height per maize plant was determined during leaf stage seven.

Five common foliar diseases were scored on plot basis. The diseases included *Curvularia* leaf spot, southern leaf blight caused by *H. maydis*, northern leaf blight caused by *H. turcicum*, maize rust caused by *P. polysora*, corn smut caused by *U. maydis* and streak caused by maize streak virus. In identifying the disease symptoms, a handbook of diseases published by the International Maize and Wheat Centre (CIMMYT) was used (CIMMYT, 2004). Severity of each of the five diseases was evaluated using rating scale of 1 to 5 according to the breeder's scale International Institute for Tropical Agriculture's standard (IITA) and Blight *H. maydis*, *H. turcicum* are scored on plot basis on a scale of 1 to 5 as given as follows; 1 = slight infection very few lesions on leaves, usually only on the lower leaves of the plant; 2 = light infection few to moderate lesions on leaves below top ear, no lesions on leaves above the top ear; 3 = moderate infection, moderate to large number of

lesions on leaves below the top ear, few lesions on leaves above the top ear; 4 = heavy infection, large number of lesions on leaves below the top ear, moderate to large number of lesions on leaves above the top ear; 5 = very heavy infection, all leaves with large number of lesions leading to premature death of the plant and light ears (Badu-Apraku et al., 2012).

Similarly, *Curvularia* leaf spot, rust (*P. polysora*), and streak were scored on plot basis using a 1 to 5 rating scale based on the proportion of the ear leaf that is covered with lesions. The scale is as follows: 1 = slight infection: less than 10% of the ear-leaf covered by lesions; 2 = light infection: 10 to 25% of the ear-leaf covered by lesions; 3 = moderate infection 26 to 50% of the ear-leaf covered by lesions; 4 = heavy infection: 51 to 75% of the ear-leaf covered by lesions, leading to premature death of the plant and light cobs; 5 = very heavy infection: 76 to 100% of the ear-leaf covered by lesions, leading to premature death of the plant and light cobs (Badu-Apraku et al., 2012).

In all cases, scores < 3 signified resistance of genotype to the disease while any score greater than 3 indicate susceptibility of the genotypes to the disease (Badu-Apraku et al., 2012). Plant aspect was scored on a plot basis using a scale of 1 to 5 based on the plant's general appeal and architecture with features such as uniform medium-height plants standing erect, strong stalk, uniformly big ears, well covered with husk and uniformly placed at the middle of the plant, no visible symptoms of any common tropical diseases on leaves, stems, and ears, on the scale, 1 = excellent plant architecture; 2 = very good plant architecture; 3 = satisfactory plant architecture; 4 = poor plant architecture and 5 = very poor plant architecture (Akinwale and Adewopo 2016). When the cobs were fully developed, the varieties were assessed for their susceptibility to root and stem lodging based on scale 1 to 5, where, 1= excellent (no lodging), 2 = very good, 3 =

good, 4 = fair and 5 = poor.

Husk cover as well ear aspects were rated visually on a scale of 1 to 5, where 1 = clean, uniform, well covered husk, deep greenish plant appearance, large and well-filled ears, and 5 = opened husk with rotten, small and partially filled ears (Badu-Apraku et al., 2012). Sixteen weeks after planting, harvesting was done. Data were recorded on the number of ears per plot. Ear aspect was measured on a plot basis using a scale of 1 to 5, where 1 = excellent ears: uniformly big ears, well filled with grains, no ear rot or other ear disease symptoms, 2 = very good ears: uniform moderate-sized ears, well filled with grains, no ear rot or other ear disease symptom; 3 = satisfactory ears: less uniform moderate-sized ears, well filled with grains, no ear rot or other ear disease symptom; 4 = poor ears: small-sized ears, poorly filled with grains, slight symptoms of ear rot and other diseases; and 5 = very poor ears: very small-sized ears, ears poorly filled with grains and severe symptoms of ear rot and other ear diseases (Badu-Apraku et al., 2012).

Cobs were harvested on plot basis and ear weight was taken using a weighing balance. Grain yield per hectare was computed on the basis of ear weight per plot, and the weight was adjusted to 80% shelling percentage (800 g grain kg⁻¹ ear weight) and 15% (150 g kg⁻¹) moisture content (Badu-Apraku et al., 2012).

Statistical analyses

Data collected were subjected to analyses of variance (ANOVA) to test for significant differences among genotypes for the traits measured for each year. Having tested for homogeneity of variance using Levene's test, combined ANOVA was carried out to test the effect of year, variety and variety × year interaction of the agronomic performance

Table 2. Means squares from analysis of variance for emergence and flowering traits of 40 maize varieties belonging to two maturity groups evaluated under field conditions at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria

Source	DF	Emergence (%)	Days to tasseling	Days to anthesis	Days to silking	ASI
Year (Y)	1	89.01	2.10	3.69	2.01	4.01*
Block/Rep*Y	24	169.93	1.21	2.08	1.15	1.41
Rep/Y	4	11.75*	8.01*	26.29**	26.85**	0.08
Variety (G)	39	1291.74**	3.28*	5.39**	11.28**	7.22**
Extra-early (EE)	15	435.63**	2.72	5.19**	11.12**	0.32
Early (E)	23	589.19**	3.40**	5.18**	1.90**	4.84*
E vs EE	1	144.92	5.67	16.37**	2.42	6.19*
G x Y	39	103.01	2.20	2.18	1.65	1.97
Error	69	222.15	2.01	2.37	1.27	2.25
R-square (%)		78	53	63	85	66
CV (%)		24.27	2.78	2.91	2.04	64.88

*, ** Significant and highly significant at $p < 0.05$ and $p < 0.01$ levels, respectively, DF: degree of freedom, S V: source of variation, C V: coefficient of variation, ASI: anthesis-silking interval.

Table 3. Means squares from analysis of variance for grain yield and yield component traits of 40 maize varieties belonging to two maturity groups evaluated under field conditions at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife Nigeria in 2014 and 2015.

Source of variation	DF	EPP	Number of ears per plot	Ear aspect	Plant aspect	Plant height, cm	Grain yield, kg ha ⁻¹
Year (Y)	1	0.34	10.24	0.19	0.22	0.031	1154334**
Block/Rep*Y	24	0.65	33.02	0.1	0.71*	0.024	156748.2
Rep/Y	4	0.43	390.21**	0.83**	0.53	0.071*	954093.3
Variety (G)	39	0.39	167.14**	0.20	0.27	0.023*	1012167.9**
Extra-early (EE)	15	0.27	49.11	0.23	0.45	0.02	278276.0
Early (E)	23	0.47	97.01**	0.10	0.19	0.02	561603.6**
E vs EE	1	0.22	9.06	1.01**	0.005	0.01	86051.4
G x Y	39	0.33	51.06*	0.25	0.002	0.021	1002471.5*
Error	69	0.44	62.4	0.14	0.32	0.02	335135.5
R-square (%)		67	65	53	46	57	69
C.V (%)		26.53	29.18	13.03	18.9	7.51	23.51

*, ** Significant and highly significant at $p < 0.05$ and $p < 0.01$ levels, respectively.

and disease scores.

Significant means were separated using Least Significant Difference (LSD). Correlation and regression analyses were also done to assess relationship among traits. All analyses were carried out using Statistical Analysis Software (SAS) version 9.2 (SAS Institute, 2002).

RESULTS AND DISCUSSION

Field performance of the 40 early and extra-early maize varieties

Results of analysis of variance on the response of the 40

newly developed varieties of maize to some common tropical diseases revealed that, the 40 varieties were significantly different from flowering traits (Table 2), as well as for yield and yield components except EPP ear aspect and plant aspect (Table 3). For disease scores, the varieties were not significantly different except for *Helminthosporium maydis* (Table 4). Partitioning the variety effect into variation within varieties in each maturity group and variation between the two maturity groups revealed that significant variation among the 40 varieties for emergence and days to silking was due to the variation in varieties within each maturity group rather than variation between maturity groups. Furthermore,

Table 4. Mean squares from analysis of variance for disease severity scores of the 40 maize varieties belong to early and extra-early maize maturity groups tested under field conditions at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife in 2014 and 2015.

Source of variation	DF	<i>Curvularia</i> Leaf spot	<i>Exserohilium</i> <i>turcicum</i>	Maize streak	<i>Helminthosporum</i> <i>maydis</i>	<i>Puccinia polysora</i> RUST	<i>Ustilago maydis</i> (SMUT)
Year (Y)	1	0.11	0.06	0.21	0.12	2.12	0.0021*
Block/Rep*Y	24	0.1	0.70*	0.27	0.47	0.72**	0.0002
Rep/Y	4	1.28**	4.83**	0.13	0.47	9.64**	0.0001
Variety (G)	39	0.09	0.21	0.27	0.53*	0.21	0.0010
Extra-early (EE)	15	0.10	0.22	0.14	0.57	0.18	0.0053
Early (E)	23	0.17	0.27	0.33	0.54*	0.44	0.0030
E vs EE	1	0.02	0.31	0.02	1.19	0.02	0.0021
G × Y	39	0.05	0.23	0.27	0.73*	0.21	0.0030
Error	69	0.08	0.23	0.31	0.3	0.26	0.0003
R-square (%)		55	60	43	54	66	42
CV (%)		8.25	20.36	43.00	24.37	16.13	6.38

*, ** Significant and highly significant at $p < 0.05$ and $p < 0.01$ levels respectively, S V: source of variation, DF: degree of freedom. CV: coefficient of variation.

variation in the 40 genotypes was accounted for by significant variation among varieties within early maturity group alone, for anthesis-silking interval (ASI) was as a result of variation within early varieties and between the two maturity groups while for days to anthesis, the difference among the 40 genotypes was due to variation among varieties within each and between maturity groups (Table 2). Forty maize varieties exhibited resistance to smut (*U. maydis*), southern leaf blight (*H. maydis*), northern leaf blight (*Exserohilium turcicum*) and streak disease as indicated by their low maximum severity scores but susceptible to *Curvularia* leaf spot (*C. lunata*) and leaf rust (*P. polysora*).

The result of this study on the response of 40 maize varieties to *H. maydis* was contrary to findings in earlier studies where, the organism

caused negative effect on maize genotypes having male sterility inducing T cytoplasm (Gengenbach et al., 1973; Earle et al., 1978). In these studies, trace of the pathogen caused epiphytomy on maize hybrids which have been produced on the basis of Texas type of sterile cytoplasm. The result stimulated further studies on developing alternative types of male sterility inducing cytoplasm in different crops. However, in this study, experimental varieties were used, not cytoplasmic male sterility (CMS) hybrids and this may explain differences in the response of the genetic materials to the pathogen.

The varieties were significantly different for most traits measured. All varieties had desirable scores (maximum scores < 3.0) for streak and smut, indicating that all varieties showed resistance to both diseases. In contrast, the maximum scores

for the varieties were greater than 3.0 for *E. turcicum*, *Curvularia* leaf spot, *H. maydis* and rust fungus, indicating that at least one variety was susceptible to the fungal diseases (Table 5).

DTE STR-W SYN POP C₃F and 2013 DTE STR-Y SYN F₁ had the highest yield, 2840 and 2832 kg ha⁻¹, respectively (Table 5). The two varieties had desirable scores for most diseases (<3.0) except for *Curvularia* and rust (Table 5). This implied that even though the two varieties had high symptoms of *Curvularia* leaf spot and leaf rust fungi, the fungi infection did not affect the yielding ability of the two highest yielding varieties. However, it is not advisable for breeders to wait until these two diseases get beyond the economic threshold before attention is paid to upgrade tolerance of the newly developed varieties.

For streak and smut, 100% of the varieties were

Table 5. Means for disease severity scores and other agronomic traits of the top 10 yielding varieties and 5 worst yielding varieties at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria.

Variety	Maturity	Emergence	Days to tasseling	Days to anthesis	Days to silk	ASI	<i>E. turcicum</i>	<i>Curvularia</i>	Streak	<i>H. maydis</i>	Smut	Rust	Ear aspect	Plant aspect	Ears per plot	Ears per plant	Grain yield
DTE STR-W syn POP C ₃ F	Early	60	53	55	58	2	3.0	3.3	1.2	2.2	1.0	3.5	2.8	3.3	33.6	1.5	2840
2013 DTE STR-Y syn F ₁	Early	55	53	56	60	4	2.8	3.1	1.0	2.4	1.0	3.0	2.8	3.2	27.1	1.0	2832
TZEE-WPOP STR QPM C ₂	Extra-early	46	53	56	61	5	3.2	3.3	1.0	2.5	1.0	3.2	3.2	2.8	32.5	1.2	2703
TZE-W POP DT STR C ₄	Early	20	56	58	59	1	3.0	3.6	1.6	2.7	1.0	3.1	3.0	2.4	34.5	2.3	2669
TZEE-W POP STR 104 BC ₂	Extra-early	56	54	56	59	3	3.1	3.5	1.0	2.8	1.2	3.5	2.9	2.9	29.3	1.0	2637
2004 TZEE W POP STR C ₄	Extra-early	52	53	55	58	3	2.5	3.2	1.1	3.1	1.0	3.4	3.2	2.7	22.0	1.2	2629
DT-W STR syn	Early	38	54	55	60	4	2.8	3.5	1.1	2.4	1.0	3.3	3.0	2.8	34.5	1.2	2598
syn DTE STR-W	Early	37	54	56	61	5	2.7	3.5	1.1	2.7	1.0	3.0	2.7	3.0	27.9	1.7	2555
2009 DTE-Y STR	Early	35	54	58	59	1	3.2	3.4	1.3	2.3	1.0	3.2	2.7	3.3	28.5	0.9	2530
syn DTE STR-Y	Early	43	53	55	59	5	2.8	3.8	1.5	2.1	1.0	2.6	3.0	2.6	35.9	1.3	2491
2008 DTMA-Y STR	Early	54	55	59	61	2	3.3	3.3	1.7	2.9	1.0	2.9	3.1	3.3	24.6	1.1	1542
EVDT-Y 2000 STR	Early	35	53	56	60	4	3.4	3.5	1.2	2.3	1.0	3.0	2.9	2.8	25.4	1.4	1489
TZEY Pop DT STR QPM	Early	14	54	56	60	3	2.9	3.8	1.1	2.7	1.0	2.7	2.7	3.3	19.4	0.8	1427
EVDT-W 2008 STR	Early	40	54	55	60	4	2.8	3.2	1.4	2.3	1.0	3.0	3.0	3.0	29.2	1.3	1414
2008 TZEE Y STR	Extra-early	57	53	54	56	2	2.7	3.4	2.2	1.9	1.0	3.3	3.0	2.4	26.4	1.0	1373
Mean		41	54	56	60	3	2.9	3.3	1.3	2.3	1.0	3.0	2.9	3.0	27.3	1.2	2113
SE		2.25	0.17	0.23	0.22	0.19	0.05	0.04	0.06	0.07	0.01	0.06	0.04	0.05	0.80	0.05	65.0
Minimum		10	53	54	56	1	2.1	2.7	0.9	1.2	1.0	2.1	2.4	2.4	16.1	0.6	1373
Maximum		61	57	61	64	5	3.4	3.8	2.4	3.1	1.2	3.7	3.6	3.6	35.9	2.3	2840

tolerant (Table 6). Disease with highest percentage of susceptible varieties was *Curvularia* leaf spot (90%), followed by rust (82.5%) Northern corn blight (22.5%) and Southern corn blight (2.5%). It is important to note that Northern corn blight, which is known as a common disease in higher altitude and colder regions is becoming prominent in the hotter and humid climate. The reason for this is yet to be fully investigated. However, the scenario could be attributed to climate change. Furthermore, it was observed that 90 % of the 40 varieties were susceptible to *Curvularia* leaf spot. Of these 40 varieties, 100% of the extra-early maize varieties were

susceptible while 83% of the early varieties showed susceptibility to *Curvularia* (Table 6). This suggests that more of the extra-early varieties were susceptible to *Curvularia*. Similarly, more of the extra-early varieties showed susceptibility to leaf rust (69%) than the early varieties (54%). In contrast, the early maize had more varieties that were susceptible to Northern corn blight (25%) than the early maize (19%) (Table 6).

Relationship among traits

Across maturity groups, northern corn blight,

southern corn blight, streak and smut had no significant relationship with any agronomic traits including grain yield (Table 7). This result may suggest that even though there were visible symptoms of these diseases on the plants, they did not significantly affect the performance and productivity of the maize varieties, thus most of the varieties evaluated, by and large, showed tolerance to most common diseases. This result is in agreement with Olakojo et al. (2005), who reported tolerance of newly developed QPM and normal-endosperm maize to some diseases in south-western Nigeria. The result on the effect of streak is in contrast with the findings of Bosque et

Table 6. Proportion of tolerant and susceptible varieties for different diseases at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria.

Disease	All varieties		Early varieties		Extra-early varieties	
	Tolerant (%)	Susceptible (%)	Tolerant (%)	Susceptible (%)	Tolerant (%)	Susceptible (%)
<i>Curvularia</i> leaf spot	10.0	90.0	17	83	0	100
Northern corn blight	77.5	22.5	75	25	81	19
Maize streak	100.0	0.0	100	0	100	0
Southern corn blight	97.5	2.5	100	0	94	6
Corn smut	100.0	0.0	100	0	100	0
Leaf rust	17.5	82.5	46	54	31	69

Table 7. Correlation between agronomic traits and severity scores of common diseases across 40 early and extra-early maturing maize varieties and for each maturity group at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria.

Variables	Combined						Early						Extra-early					
	TUR	MAYDIS	CUR	SR	RUST	SMUT	TUR	MAYDIS	CUR	SR	RUST	SMUT	TUR	MAYDIS	CUR	SR	RUST	SMUT
TASSEL	-0.04	0.14	-0.59**	-0.13	-0.33*	0.17	-0.17	0.22	-0.46*	-0.12	-0.35	0.08	0.19	0.06	-0.77**	-0.31	-0.42	0.33
ANTH	-0.04	0.14	-0.67**	-0.22	-0.10	0.06	-0.25	0.20	-0.56**	-0.30	-0.13	0.12	0.28	0.15	-0.82**	-0.29	-0.20	0.08
SILK	0.09	0.04	-0.52**	-0.10	-0.28	-0.02	-0.14	-0.06	-0.23	-0.05	-0.60**	-0.12	0.25	0.16	-0.67**	-0.27	-0.17	0.04
EASP	-0.04	0.03	0.00	-0.18	-0.05	-0.11	-0.21	0.03	0.30	-0.14	0.04	-0.18	0.07	-0.05	-0.21	-0.14	0.00	-0.15
PASP	-0.07	-0.05	-0.13	-0.28	0.09	-0.14	-0.15	-0.02	-0.23	-0.51**	0.20	-0.23	0.00	-0.12	-0.08	0.07	0.03	-0.09
EARNO	-0.07	-0.08	0.31*	0.05	0.01	-0.06	-0.24	0.21	0.27	0.01	0.29	-0.20	0.21	-0.68**	0.38	0.18	-0.48*	0.12
MC	-0.05	0.09	0.28	-0.04	-0.03	-0.19	0.20	0.15	0.63**	0.01	-0.02	-0.01	-0.33	-0.02	0.01	-0.08	0.02	-0.37
ASH1	0.15	-0.11	0.19	0.15	-0.21	-0.10	0.17	-0.24	0.44*	0.28	-0.24	-0.19	0.09	0.09	-0.13	-0.10	-0.04	-0.04
EPP	-0.09	0.16	0.19	-0.12	-0.15	-0.03	0.01	0.43*	0.23	-0.18	-0.07	-0.07	-0.25	-0.38	0.16	-0.02	-0.37	0.07
YIELD	-0.04	0.04	-0.06	-0.08	0.12	0.10	0.01	-0.06	-0.06	0.04	0.20	-0.07	-0.10	0.29	-0.05	-0.52*	-0.07	0.36

al. (1998), who reported that streak mosaic virus disease was negatively correlated with plant height, dry weight, grain weight per plot, 1000-grain weight, ear length and diameter. This confirms that, maize breeders in this sub-region routinely incorporate tolerance/resistance to some common diseases into newly developed varieties even when the breeding target is not on disease

resistances. More so, the result on *E. turcicum* was contrary to the findings of Nwanosike et al. (2015) who reported in their work on 5 varieties of maize that, Northern corn blight was negatively correlated with yield grain. Contrary to the response of the maize plants to the diseases mentioned above, *Curvularia* had significant correlation with days to tasseling ($r = -$

0.59 **), days to anthesis ($r = - 0.67 **$), days to silk ($r = - 0.52 **$) and number of ears per plot ($r = 0.31 *$). This result indicates that as scores for *Curvularia* increased (indicating susceptibility) the days to flower decreased (earliness). In other words, *Curvularia* infection resulted in earliness to flower or the early maturing varieties which is more susceptible to *Curvularia* infection than the

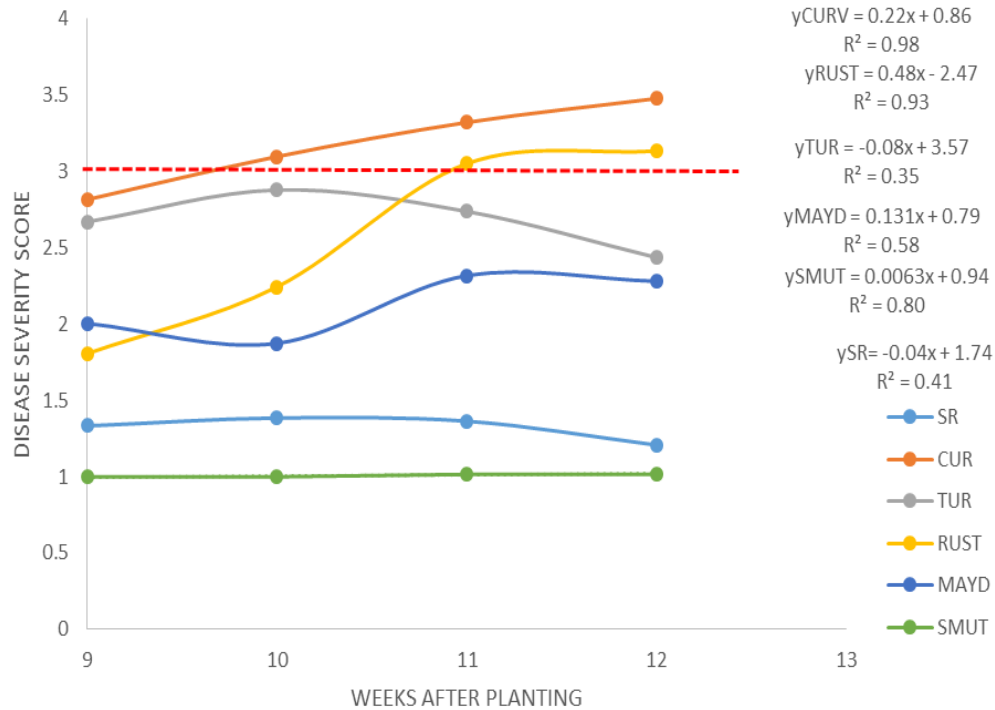


Figure 1. Response of extra-early maize varieties to common diseases in the humid rainforest agro-ecological conditions at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria. SR = Maize streak; CUR= Curvularia leaf spot; TUR = Northern corn blight by *Exserohilium turcicum*; RUST= Rust fungi; corn smut *Ustilago maydis*. MAYD= Southern corn blight caused by *Helminthosporium maydis*

late maturing ones. Varieties that were susceptible to *Curvularia* flowered earlier than the tolerant varieties. In a study on incidence and severity of some common diseases of maize, Akonda et al. (2015) reported that *Curvularia* leaf spot was one of the two most virulent diseases in the region negatively affecting plant's health and yield. In addition, leaf rust also had significant correlation with days to tasseling ($r = -0.33^*$), although the r^2 of 10.89% indicates that the relationship is very weak.

Furthermore, results of correlation between agronomic traits and diseases severity scores showed differential pattern in the response of the the different maturity groups to the different diseases. No agronomic traits had significant correlation with severity scores for *E. turcicum* and smut, indicating that these diseases had no significant effect on the performance and productivity of both maturity classes of maize. Among phenological traits, *Curvularia* leaf spot had significant relationship with days to tassel ($r = -0.46^*$), days to anthesis ($r = -0.56^*$) and ASI ($r = -0.44^*$) for early maize but for extra-early maize, *Curvularia* score had significant correlation with days to tassel ($r = -0.77^{**}$), days to anthesis ($r = -0.82^{**}$) and days to silk ($r = -0.67^{**}$). This result implies that *Curvularia* significantly increase days to flowering of maize. Since the correlation coefficient and resulting R-

squares between *Curvularia* and flowering traits were higher for extra-early maize than those of early maize, it indicates that *Curvularia* had higher effect on flowering traits of extra-early than early maize varieties. Moreover, *H. maydis* had significant relationship with number of ears per plant (EPP) among early maize varieties but had significant relationship with number of ears per plot among extra-early maize varieties.

Results of the regression analysis revealed that only rust and *Curvularia* leaf spot scores got beyond the susceptibility threshold (>3.0) for extra-early maize varieties (Figure 1). This implies that proper management practices are necessary to bring these diseases under control when extra-early maize varieties are produced. In addition, the results further showed that rust had the highest rate of disease progression per week (b-value = 0.48) followed by *Curvularia* leaf spot (b-value = 0.22). In contrast, other diseases were below the susceptibility threshold with smut and streak being the lowest. This implied that extra-early maize varieties are still largely resistant to diseases such as smut, streak, Northern and Southern leaf blight and therefore no need for control measures.

The pattern of response of early maize varieties to the common diseases under field conditions was similar to that of extra-early varieties. For the early, only *Curvularia*

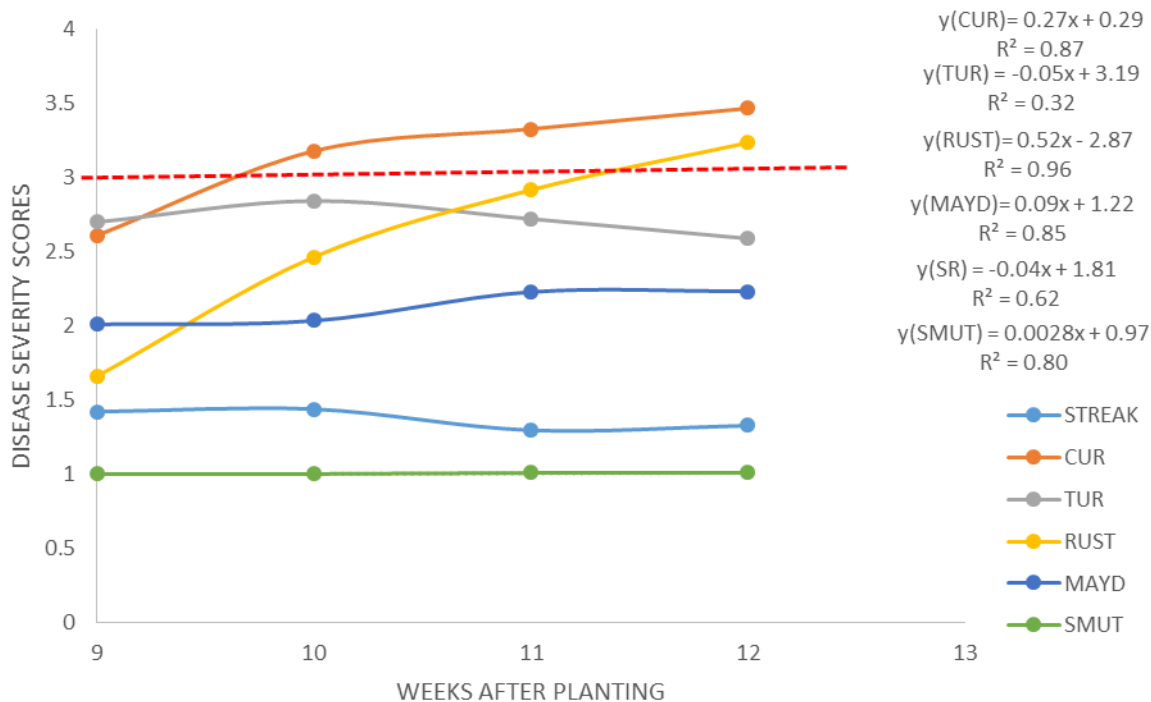


Figure 2. Response of early maize varieties to common diseases in the humid rainforest agro-ecological conditions at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria. SR = Maize streak; CUR= *Curvularia* leaf spot; TUR = Northern corn blight by *Exserohilium turcicum*; RUST= Rust fungi; corn smut *Ustilago maydis*. MAYD= Southern corn blight caused by *Helminthosporium maydis*.

leaf spot and leaf rust exceeded the susceptibility threshold, leaf rust had highest value for disease progression (b-value = 0.52), followed by *Curvularia* leaf spot (b-value = 0.27) (Figure 2), a trend similar to that of the extra-early varieties. This result implies that the early maturing maize varieties were also sensitive to these two diseases and attention should be given to manage them. Apart from the two diseases, *E. turcicum* incidence was the next disease, fast approaching the threshold line. This disease has been reported to be a serious one, which causes huge economic damage in the high altitude regions (Yeshitila, 2003). It is therefore note-worthy to find out in this study that its incidence in low altitude climate was higher than that of Southern blight.

There is limited information on the appropriate time toward score diseases for the purpose of selecting tolerant genotypes under field conditions. The result also revealed that different diseases reached their peak at different time, suggesting that for extra-early maize, different diseases should be recorded at different times. *Curvularia* leaf spot and leaf rust, which were the diseases that reached the threshold, touched the line at different time. *Curvularia* leaf spot curve touched the threshold line shortly before 10 weeks after planting (WAP), suggesting that tolerance to *Curvularia* leaf spot among extra-early maize is better detected as from 10 WAP while tolerance to rust is best scored as from 11

WAP (Figure 1). For early maize, the two diseases which reached threshold touched the threshold line at different time, suggesting that scoring the diseases should be at different times. Following from this, *Curvularia* leaf reached the threshold line before 10 WAP and that the disease should be scored for early maize anytime from 10 WAP. For rust score, curve touched the threshold between 11 and 12 WAP, implying that the disease scoring should be scored at that time (Figure 2). The result which revealed the best time to score leaf rust under field conditions was not in agreement with that recommended by CIMMYT Maize Program (2004), who reported that the best time to score *Puccinia sorghi* is before tasseling. The extra-early maize in this study started tasseling at 6-7 WAP while early maize started tasseling at 7-8 WAP.

Due to the fact that the evaluation in this study was conducted under field condition, spread of inoculation might not be even and this may affect the result. Thus, a greenhouse study where artificial inoculation of the genetic materials is carried out might be necessary to ascertain the level of resistance/tolerance present in the new germplasm.

Furthermore, when studying the resistance of a crop to pathogen(s), it would be very useful to present information on race composition of the pathogens on a given territory. This information is not available in the

rain-forest agro-ecological zone of Nigeria. Therefore, subsequent studies should be conducted to provide this information.

Conclusion

The disease progression became severe at eight weeks after planting with visible symptoms. These symptoms increased drastically with time but all forty maize varieties still maintained their tolerance level against streak, Northern leaf blight, Southern leaf blight and smut. Although, none of these diseases significantly reduced yield, scores for *Curvularia* leaf spot and rust disease significantly exceed the resistance threshold suggesting that management of the two diseases need attention to control them before they start causing economic damage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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