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Assessing the effectiveness of nonwoven fabric pollination tents for improved grass breeding

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The practices of using isolation and distance in the seed production of open pollinated crops are fundamental concepts to ensure seed purity. We uniquely examined the effectiveness of replacing isolation plots for seed production and grass breeding with different sizes of novel nonwoven synthetic fabric pollination control tents (PCTs). Two fabrics, DWB10 and DWB24, were used along with multiple genotypes of tall fescue at Ardmore, Burneyville and Gene Autry locations in Oklahoma, USA during 2018 and 2019. Treatment effects were consistently significant in both years, but location differences were more pronounced in 2019. Interactions of treatments with locations or genotypes were not predominant. The two tent fabrics, generally, performed equally well for various traits in both years. Tent performance for both fabrics was particularly superior over control for various traits in 2019 (e.g., DWB10 tent showed a 36% increase for seed yield (SY) over the control). Introduction of fans in tents for increasing pollen flow in 2019 was not advantageous as it reduced the SY by 23%. The average temperature within tents was higher with lower average humidity than the control producing a microclimate for good yield and disease free seeds. The final germination (%) of seeds from tents and controls at 21 days was high and not much different with a minimum overall germination of 89% at Burneyville in 2018. There was no evidence of pollen contamination from tetraploid ryegrass pollen in any of the tent fabrics. Bad weather in 2018 affected the sturdiness of tents, but modifications in 2019 corrected all such mishaps. Further improvements in the structures, design and cover have since been made for field exploitation of technology in grass improvement and seed multiplication.

Key words: Ryegrass, fescue grass, pollination control tents, nonwoven fabrics.

INTRODUCTION

The *Festuca* (fescue) genus (2n=6x=42) is closely related to diploid (2n=14) ryegrass (*Lolium*) with plant taxonomists having moved several species from the genus *Festuca*, including the grasses tall fescue and meadow fescue, to the genus *Lolium*. The wide range of uses for fescues and ryegrasses vary from ornamental and turf to highly nutritious pasture for haying and grazing livestock (Darbyshire and Pavlick, 2012). These grasses

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can also be used in soil erosion control programs. There are a large range of grass cultivars derived from these genera leading to the production of substantial amounts of certified seed annually.

The majority of grasses are cross-pollinated by wind and are largely self-incompatible thus preventing their self-pollination. Individual plants in populations are highly heterozygous being hybrids of hypothetical parents. However, natural populations have substantial additive genetic variation for selection to be effective for most agronomic traits (Vogel et al., 1989). Individual plant selections, usually in thickly seeded stands or swards as forages or turf grasses, can be impractical and as such, evaluation and selection are often practiced in space-planted nurseries or small sward plots. The most effective breeding programs in forage grasses limit hand emasculation or crossing, instead utilizing recurrent selection improvement systems, which have the added benefit of retaining genetic variation in the populations. This system is continued with the random mating of selected individuals in isolated polycross nurseries to produce progenies for the next cycle of selection (Brown et al., 2014). The selected polycross progeny are then used in the development of synthetic populations. In the UK, hand emasculated pair-wise crosses between two species *Lolium multiflorum* and *Lolium perenne* are being used to make synthetic varieties from the interspecific hybrid, *Lolium boucheanum*. Grass breeders are now more interested in these types of hybrids, exploiting the higher heterosis of F1 hybrid varieties between two or more compatible interspecific combinations vs. more narrow based synthetic varieties. This approach, though more productive, requires modifications in the ways interspecific varieties are composed and subsequently multiplied.

Wind pollination is related to the size of the grass pollen being distributed since neither very large nor very small grains are wind pollinated. Grass pollen is divided into two types based on grain size; wild grass types range from 25 to 35 µ (with exceptions of 35 or 40 microns) and the cultivated grasses range from 35 to 50 µ with the modal peak at 40 µ (Erdtman, 1943). According to Wodehouse (1935), *Lolium* pollen grain size ranges from 22 to 33 µ. Geisler (1945) reported a range of 24-39 µ with modal peak of 31 µ for a group of six grass species including *Poa* and *Festuca*.

Pollination bags, isolation plots, isolation chambers and pollination control tents (PCTs) are some of the methods used in the controlled crossing of grasses. Pollination bags are only useful on a limited scale. Isolation chambers are expensive to build and run in order to maintain a controlled microclimate, and isolation plots demand very long distances between plots of the same species (305 m for Breeder Seed) thus limiting the number of entries to be multiplied. However, research on the effectiveness of PCTs is limited. This study addresses this gap by evaluating novel PCT technologies in a grass breeding program using nonwoven, re-usable synthetic tent fabrics.

The aim of this study is to examine the possibility of substituting the use of isolation and distance in small field crossing or seed production nurseries with new PCTs and extending our knowledge about the microclimate within such structures in order to obtain a healthy and high seed set while at the same time providing pollen proofing. Consequently, it lays the foundation for new research in plant breeding, investigating novel options that are potent enough to increase the efficiency of breeding operations in all crops by enabling many crosses to be made simultaneously or by increasing the number of seed multiplications of promising populations. The major objectives of the study were: (1) evaluating PCT structures for robustness, durability and strength of cover fabric materials under field conditions, (2) testing the pollen proofing ability of fabric materials, (3) comparing the microclimate within PCTs with outside control conditions, and (4) assessing the comparative seed output of healthy seeds and plant performance for biological traits.

MATERIALS AND METHODS

Experimental sites

Three environmental sites, located in southern Oklahoma, USA on Noble Research Institute, LLC farms, which varied in humidity, temperature, windiness, minor elevation difference and soil type were chosen for PCT testing by placing one set of PCTs at each site. The first site was in Ardmore on the research park farm (34.10° N, 97.10° W; elevation 266 m) on Heiden clay (fine, montmorillonitic, thermic Udic Chromusterts). The second site was in Gene Autry on the Dupy farm (34.17°N, 96.58° W; elevation 223 m) on Dale silt loam (fine-silty, mixed, superactive, thermic Pachic Haplustoll). The final site was located in Burneyville on the Red River farm (33.53°N, 97.15° W; elevation 221 m) on Eufaula loamy sand (siliceous, thermic Psammentic Paleustalfs). The distance of sites ranges from 5 to 56 km from the Noble Research Institute's main campus.

PCT types

DWB010 and DWB24 pollination tents with different fabric materials were used in the present study and were obtained from PBS International, UK. These materials were used in order to allow better air permeability, as they are more open compared to the regular control counterpart Duraweb® (Hayes and Virk, 2016). However, their architecture and fibre shape hinders pollen grain transmission by creating a more difficult passage through the fabric. The following are the major features of the fabrics.

DWB10

Nonwoven spun-bound polyester; thickness (mm) 0.33; mass per unit area/weight (gm^{-2}) 100; air permeability ($l/m^2/s$) 550; light transmission (% 350 - 800 nm wavelength) 35.5; maximum pore size (microns) 152; fibre cross section is simple. It has waxier surface than DWB24.

Figure 1. Large pollination control tent (PCT) set up (left) 2019 and Research Associate Dusty Pittman setting the frame and doing some final weeding and plant care before the cover is placed over the PVC frame of small tent in 2019. A soaker hose was placed inside the tent frame for watering the plants without disturbing the cover during pollination process.

DWB24

Nonwoven spun-bound polyester; thickness (mm) 0.40; mass per unit area/weight (gm^{-2}) 110; air permeability (l/m^2/s) 1470; light transmission (% 350 - 800 nm wavelength) 39; maximum pore size (microns) 214; fibre cross section is complex.
The following types of PCTs were used:

(a) Small PCTs tested in 2018 and 2019
- Three small PCTs, DWB10, size 1.5 m × 3 m × 2 m
- Three small PCTs, DWB24, size 1.5 m × 3 m × 2 m)

(b) Large PCTs tested in 2019
- One large PCT, DWB10, size 6 m × 6 m × 2 m
- One large PCT, DWB24, size 3 m × 12 m × 2 m).

Design

The frames of PCTs were made of PVC piping and were secured to the ground by placing two sand bags (22.6 kg) on each side on the bottom pipe of the frame. The structures were rigid once assembled. Additional dirt was placed around all sides of the PCT when it rained because the dirt tended to settle or was washed away, exposing the skirt edges. Soaker hoses were supplied to each tent for supplementary irrigation, if needed. Covers of both types of fabric fitted snuggly on the appropriate frames (Figure 1).

Smaller PCTs used in 2018 were stored for re-use in 2019. Both types of fabric were washed using a solution of Clorox® bleach (10%) and distilled water before re-use to clean and remove any contaminants. Duct tape was used on any fabric seams of the PCTs (both fabrics) to make minor repairs.

Small PCTs

During 2018 and 2019, two smaller PCTs, DWB10 and DWB24 were placed at each of three sites. Within each PCT, 15 tall fescue plants (*Lolium arundinaceum* (Scherb.) Darbyshire) representing three genotypes and cloned five times each were transplanted and grown.

A control isolation group (open pollinated) was planted at each location containing 15 tall fescue plants (same 3 genotypes used in tents and cloned 5 times each = 15 plants) at a minimum of 305 m away, which is the minimum distance between breeder or foundation seed increase of tall fescue as recommended by the Seed Certification Service in Oregon, USA (Oregon Seed Certification Service Handbook, 2018).

In the 2019 trial, an additional control was added at each location. This 'open control' of 15 tall fescue plants (open pollinated) was located at least 305 m from the PCTs and the other control group of tall fescue plants.

Perennial ryegrass plants (*Lolium perenne* L.; 2n=4x=28) were planted around each PCT in 2018 (4 per side) to act as "pollen donors" for testing contamination in the PCTs, if any. Both species, inside and outside the PCTs, are out crossing and can hybridize (that is, *Festulolium*) allowing for detection of any chromosomal recombination between the tetraploid ryegrass and the hexaploid tall fescue resulting from pollen contamination. It was determined that approximately 15 plants around the PCT would generate enough pollen pressure with particular concentration to the southwest direction due to prevailing winds. All plants were grown and vernalized to induce flowering in early summer.

In 2018, PCTs were set up on 18th May at Ardmore and Gene Autry, and on 21st May at Burneyville. All plants were at the $E_3 – R_0$ growth stage (Moore et al., 1991) when transplanted in the field on 9-11th April in 2018 and the 22nd April 2019 (Figure 1). All tents at all locations were removed on 25th June 2018. In 2019, small PCTs were erected on 4th June and taken down on 5th July at all three locations.

Increasing pollen flow in small PCTs in 2019

Overall, seed yields in 2018 were low across all treatments and especially inside the PCTs. It was thought that airflow within PCTs may be restricted compared with the natural environment. It was hypothesized that increasing the air circulation and hence the mobility of pollen within the PCT would aid cross-pollination and improve seed yield. Therefore, portable electric fans powered by solar panels were placed in all treatments, that is, small PCTs, control, and open control groups at Ardmore and Gene Autry locations in 2019 experiments. Fans were also placed in the middle of the open control at two locations (Table 1). However, no fans were placed in any treatment at the Burneyville site. Fans were easy to setup and there was no issue with them or the solar panels in the PCTs or the field (control or open plants). The fan effect was estimated through an analysis of variance in which locations are confounded with blocks.

Larger PCTs 2019

One PCT was placed at each location. The 6 m × 6 m PCT (DWB10)

Table 1. Randomized plan of treatments with fan and no fan provisions for small tents at three sites in Oklahoma, USA.

Ardmore	Gene Autry	Burneyville
Tent DWB10, Fan	Open, Fan	Tent DWB24, no fan
Tent DWB24, Fan	Tent DWB10, Fan	Control, no fan
Control, Fan	Control, Fan	DWB10, no fan
Open, Fan	Tent DWB24, Fan	Open, no fan

Figure 2. An Onset HOBO data logger inside of a pollen control tent.

was placed at the Unit 3 Farm (34.11° N, 97.05° W; elevation 248 m) on a Windthorst fine sandy loam (fine, mixed, active, thermic Udic Paleustalf) and the 3 m × 12 m tent (DWB24) was erected at the Headquarters Farm (34.08° N, 97.12° W; elevation 252 m) on a Heiden clay (fine, montmorillonitic, thermic Udic Chromusterts). Both of these sites were located in Ardmore. Each tent contained 40 tall fescue plants (4 genotypes cloned 10 times each = 40). A control isolation (open pollinated) was planted at each location containing 40 tall fescue plants (same 4 genotypes used in the PCTs cloned 10 times each = 40) at a minimum of 305 m away. As in the small PCTs, all plants were grown and vernalized to stimulate flowering in early summer. PCTs were set up on 5th June at both locations. Both the inside and outside plants were harvested on 5th July and tents taken down on 6th July 2019.

Microclimate assessment

A HOBO MX temperature and relative humidity data logger (Onset Computer Corporation, Inc.) was placed in each PCT by suspending them from the roof (Figure 2). These data loggers are battery powered and record temperature, relative humidity and the dew point. Since the loggers have Bluetooth connection, we were able to collect data without disturbing the environment in the PCT.

Weather data were also collected from weather stations (Mesonet.org) located on the Noble Research Institute farms for comparison for the duration of the trial period. The same parameters were collected, along with the average maximum wind speed and maximum wind gusts, at each site.

Data collection

Biological traits

(1) After pollination and seed set, data on a number of biological traits were collected: plant height (cm) = PH and growth habit (1 = decumbent, 2 = semi-erect, 9 = fully erect scale) = GH. A visual disease score (1 to 5; 0 = no disease, 5 = death due to disease) = DS was also given to each plant.
(2) The seed related data were collected on: seed yield per plant (g) = SY, 1000-seed weight (mg) = SW, the presence of ergot (%), a visual seed quality score (1 to 5; 1= excellent, 5 = extremely poor) = SQ and germination rates on 7, 14 and 21 on the basis of 100 seeds from a sub-set of plants from each tent.
(3) The harvested seeds from all of the plants individually inside the PCTs were collected and assessed for various traits (Table 2).

Germination rate (%)

For germination rate 100 seeds per clone were sown for each genotype in treatments. The germinated seeds were counted on 7, 14 and 21 days after sowing to record percent germination.

Pollen contamination

For 2018, the measurement of contamination from ryegrass pollen from outside the PCTs on to the tall fescue plants was assessed by

Table 2. Summary of experimental details of pollination control tent (PCT) trials conducted on tall fescue in 2018 and 2019.

Tent size	Description	2018	2019
Small	Locations	3= Ardmore, Burneyville, Gene Autry	3= Ardmore, Burneyville, Gene Autry
	Treatments	3= DWB10, DWB24, Control	4=DWB10, DWB24, Control, Open
	Genotypes	3 = Geno 1, 2, 3	3= Geno 1, 2, 3
	Biological traits	PH, GH, SY, SW, DS, Ergot, SQ	PH, GH, SY, SW, DS, Ergot, SQ
	Germination (%)	7, 14, 21 days count	7, 14, 21 days count
	Fan effect	-	Fan in Burneyville but in other two locations Traits and germination count recorded
Large	Locations	-	2= HQ Farm, Unit 3 Farm
	Treatments	-	3= DWB10, DWB24 and control
	Genotype	-	4 with 10 replicate plants in each
	Biological traits	-	As in 2018
	Germination (%)	-	As in 2018

PH= plant height (cm), GH= Growth habit (1-9 scale), SY= seed yield per plant (g), SW= 1000-seed weight (mg), DS = Disease Score (1 to 5 scale), Ergot (%), and SQ = Seed Quality Score.

Figure 3. Photograph of the type of wax-coated paper bags that were used to test for pollen contamination in the field compared to the DWB10 and DWB24 bags provided.

looking at the possibility of hybridization of the perennial ryegrass and tall fescue. The hybrids are generally sterile and though fertility can be restored by chromosome doubling, such plants are unstable and experience chromosome loss (Scott and White, 1988). We examined some chromosomal pairing in the hybrids of the ryegrass and tall fescue.

To measure any contamination from outside pollen at the Research Park farm at Ardmore in the 2019 small PCT trials, an existing tall fescue population was chosen to measure for any pollen contamination due to the fabric (different nursery than the control and open nurseries at this site). Sixty plants were bagged (20 with DWB10 bags, 20 with DWB24 bags and 20 with wax coated paper bags). The type of wax-coated paper crossing bags (Lawson Bag Co.) that were used is represented in Figure 3. Plants were bagged between June 1 and 5th. The bags were sealed with weather proof tape and wooden stakes were used to support the tiller and bag in the field during the test period. The remaining heads in the plot were allowed to cross-pollinate. Bags were removed during the July 5-8 time-period. At this time, the bagged panicle was harvested. To determine if any seed were produced, panicles were later conditioned on a rubbing board. A total of four of the wax-coated bags were lost due to the weather. None of the

Table 3. Pollen proofing evaluation using small bags for individual panicles, 2019.

Bag type	Bags with seeds detected	Bags with no seeds detected	Bags lost	Total
DWB 10	0	20	0	20
DWB 24	0	20	0	20
Paper	1	15	4	20

other bag types was lost.

Statistical analysis of biological traits recorded on individual plants and germination percent per clone was performed following analysis of variance technique described by Sokal and Rahlf (2011). Fisher's Least Significant Difference (LSD) was used for pair-wise comparison of treatment means and significantly different means were labelled with different letters.

RESULTS

The first year with the PCTs (2018) was more of a learning experience as to how to assemble the PCTs, how they withstand the weather and how to take corrective measures. For instance, at Gene Autry (Dupy farm) on the evening of 31st May, covers of both small PCTs were blown about 200 to 300 m from their original location during a thunderstorm, which produced a wind gust of 80.14 km/h as recorded by the farm weather station located approximately 750 m from the PCTs. However, the frames remained intact. The plants inside were pollinating at the time of the failure, but we placed the tents back on their frames and continued the experiment. No damage was observed to the fabric of PCT DWB24, but we had one small tear along the seam of the DWB10 PCT along the roof, which was repaired with duct tape. Thus, the need to improve anchoring was noted.

During 2019, there was no failure from wind at any location. However, the fabric covers, having been re-used, were starting to show wear. There was no animal damage in any year at any location observed to the fabric and no fan failures occurred in 2019. The experiments in 2019 are thus more reliable for conclusions and we would lay more emphasis on these results.

Pollen proofing

During 2018, the measure of any contamination from outside pollen in the PCTs was looked at through the possibility of hybridization of the ryegrass and tall fescue. We had grown tall fescue plants inside PCTs that were surrounded by ryegrass plants. Since we do not have SSR markers to measure the rate of contamination in the progeny due to outside pollen entry into the PCT, we tried to look at some chromosomal pairing due to hybridization of the ryegrass and fescue. There was no evidence of any contamination in the progeny. However, we believe that this tedious method was not very reliable. In retrospect, fabric material in the form of small pollination bags to measure any selfing by bagging individual open pollinated plants would probably have been more reliable. This technique was selected for examining pollen contamination in the following year. Therefore, in 2019 we bagged reproductive panicles in an open pollinated plot at the Ardmore location. No seeds were produced by panicles covered by DWB10 or DWB24 bags. Of the 20 wax-coated paper bags used for comparison, 16 remained intact in the field, with only one bagged panicle producing a viable seed (Table 3). Results of the pollen study across both years showed that both the DWB10 and DWB24 tents were safe from contamination of foreign pollen from other grasses.

Microclimate within PCTs vs. weather data

In general, year 2019 was better for performance of grasses than 2018. Overall, mean seed yield per plant was higher in 2019 than in 2018. It was 15.2 g in 2019 against 11.2 g in 2018 (36% increase) at Ardmore; 20.3 g in 2019 against 11.3 g in 2018 (79% increase) at Burneyville and 19.7 g in 2019 against 10.5 g in 2018 (87% increase) at Gene Autry.

Climate factors within the PCTs along with weather data collected from the adjacent weather stations for the open pollinated controls are listed in Tables 4 and 5 for smaller PCTs in 2018 and 2019, and for larger PCTs in 2019. In 2018, the average minimum temperature inside the PCTs was higher by 2 to 8 degrees than the control at various locations, but in 2019, the outside temperature was higher at Ardmore by about 2 degrees than inside the smaller PCTs (Table 4). The average maximum temperatures were higher in both large and small PCTs compared to the controls at all sites in both years by 6 to 23°. The overall average temperatures were either equal at Burneyville in 2019 or higher in all other cases than the controls by up to 9°. It was suspected a malfunctioning data logger might have recorded some erroneous values at Burneyville in 2019. We can conclude that, in general, the temperatures within smaller PCTs had a wider range from slightly lower to slightly higher temperatures compared to control (Table 4). However, the larger PCTs in 2019 showed higher minimum temperatures by 2 to 3° at both sites (Table 5).

Table 4. Climatic data collected within the small pollination control tents (PCTs) by Onset HOBO data loggers and by local weather stations (Mesonet.org) for the open pollinated controls at each test site from May 18 to June 25 during 2018 and from June 5 to July 5 during 2019. Fans were added in tents at Ardmore and Gene Autry sites in 2019.

Year	Measure	Ardmore			Burneyville			Gene Autry		
		DWB10	DWB24	Control	DWB10	DWB24	Control	DWB10	DWB24	Control
					Temperature (°C)					
2018	Min	15	14	21	14	14	22	13	13	15
	Max	48	48	33	52	52	34	50	50	37
	Av	30	30	26	31	31	28	30	30	27
	Range	33	34	12	38	38	12	37	37	22
2019	Min	15	15	13	23	14	21	15	14	18
	Max	52	49	34	40	53	34	40	53	30
	Av	33	32	25	27	33	27	27	33	24
	Range	37	34	21	17	39	13	25	39	12
					Relative Humidity (%)					
2018	Min	17	17	49	15	12	47	16	16	29
	Max	100	100	89	100	100	88	100	100	73
	Av	72	70	71	62	61	66	71	70	69
	Range	83	83	40	85	88	41	84	84	44
2019	Min	20	6	36	23	19	39	24	26	37
	Max	100	100	100	100	100	100	100	100	100
	Av	49	53	76	53	50	72	57	54	80
	Range	80	94	64	77	81	61	76	74	63
					Wind (km/h)					
2018	Max gust			64			84			80
	Av Max			35			41			37
	Direction			SW			SSW			SW
2019	Max gust			72			70			99
	Av Max			37			35			28
	Direction			SSE			SSW			SSE
					Rainfall (mm)					
2018				49			23			32
2019				161			33			152

The maximum temperatures in larger PCTs were higher by up to 15° and the average temperature by up to 6° (Table 5).

Average minimum relative humidity values were lower in the PCTs compared to the outside controls at all three locations in 2019, but were higher at the Burneyville site in 2018. Maximum relative humidity was higher in PCTs than controls in 2018, but was consistently equal in 2019. Overall, averages for relative humidity values were variable compared to the outside controls at all three locations in two years; in 2019, they were lower in the PCTs compared to the control at all sites, but in 2018 they were lower at the Burneyville site only. The other sites showed similar results. In summary, the pattern appears that the range of temperature and relative humidity is greater inside the PCTs than outside (higher highs, lower lows), with smaller structures seeing slightly greater temperature and humidity range when compared to the larger PCTs.

There is some evidence to suggest that the PCTs made from DWB24 may record higher maximum temperatures than PCTs made from DWB10 despite being more air permeable; this may result from the more open structure, increasing light penetration in the longer wavelengths. The DWB24 also seems to have a greater range of relative humidity measurements through the day than the DWB10.

The range of average maximum gust of wind was not

Table 5. Climatic data collected within the large pollination control tents (PCTs) by Onset HOBO data loggers and by local weather station (Mesonet.org) for the open pollinated controls at both farms sites at Ardmore from June 5 to July 5 during 2019.

Measure	Parameter	Ardmore HQ farm DWB10	Ardmore Unit 3 farm DWB24	Control
Temperature (°C)	Min	16	15	13
	Max	47	49	34
	Av	30	31	25
	Range	31	34	21
Relative humidity (%)	Min	24	26	36
	Max	100	100	99
	Av	74	75	76
	Range	76	74	63
Wind (km/h)	Max gust	-	-	72
	Av gust	-	-	37
	Direction	-	-	SSE
Rainfall (mm)	Total (mm)	-	-	161

very different during the two year study ranging from 35 to 41 km/h in 2018 compared with 28 to 37 km/h in 2019 (Table 4). However, the maximum gust in 2019 was higher (up to 99 km/h) compared with 2018 (84 km/h). The direction of wind was generally SW in 2018 but SE at Ardmore and Gene Autry in 2019 (Table 4).

The average rainfall was low for the study period at all locations in 2018 with a minimum of 23 mm at Burneyville (Table 4). While it was low (33 mm) at Burneyville again in 2019 (Table 4), it was relatively higher at Ardmore (161 mm) and Gene Autry (152 mm).

Small PCTs-quantitative traits

Analysis of variance

Analysis of variance for 2018 and 2019 showed consistently significant differences among treatments for PH, SY and SW and among genotypes for GH only (Tables 6 and 7). The location effect was more pronounced in 2019 being significant for all traits, but only for GH in 2018. A significant interaction in 2018 was observed between treatments and genotypes (Table 6 and Figure 4) for seed yield, which arose from the reduced yield of genotype 1 in the DWB24 tent than in PCT DWB10. The other two genotypes did not change rank for seed yield between the two PCTs (Figure 4).

Interactions in 2019 were more pronounced for GH and SW in respect of locations vs. genotypes and treatments vs. genotypes, which are summarized in Figures 5 and 6. PCTs DWB24 and DWB10 showed cross over interaction at the Burneyville and Gene Autry locations for GH with higher values recorded at Gene Autry than at Burneyville (Figure 5). The location × genotypes interaction for GH was more pronounced for Genotype 2 at Ardmore than the other locations (Figure 5). Genotype 3 interacted significantly with PCTs due to its higher performance in PCT DWB10 and lower performance in the open treatment (Figure 5). All interactions for SW were similar to GH (Figure 6).

However, contributions of interactions SS to the total SS were very small which ranged from 0.4 to 11.4% in 2018 and from 1.1 to 12.5% in 2019 (Tables 6 and 7). On the other hand, the treatment SS was a significant contributor to the total SS in both years for most of the traits except for GH.

Mean performance

Fitted mean values for traits with significant differences were compared using Fisher's *t*-test in pairwise ways. Location means showed the highest GH score at Ardmore in both years compared to other locations, which were similar (Tables 8 and 9).

A significant location effect was also observed for PH, SY and SW in 2019 (Table 9). The mean plant height was significantly lower, but significantly higher for SW at Ardmore compared to the other two locations, which were similar. Significant mean SY differences for locations were in the order Burneyville > Gene Autry > Ardmore (Table 9). The highest mean SY at Burneyville in 2019 was accompanied with higher PH and average GH and

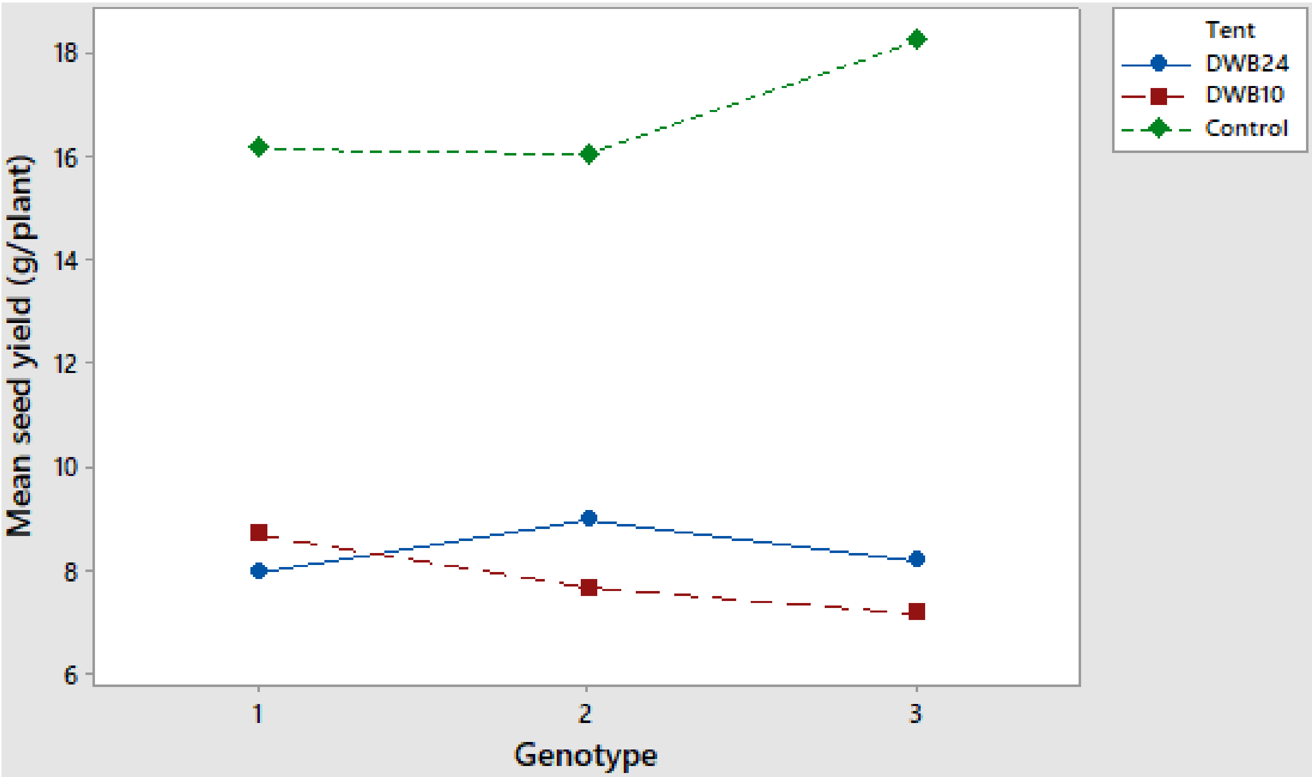

Figure 4. Interaction plot of genotypes vs. treatments (tents) for seed yield per plant (g) in small pollination control tent (PCT) trials in 2018.

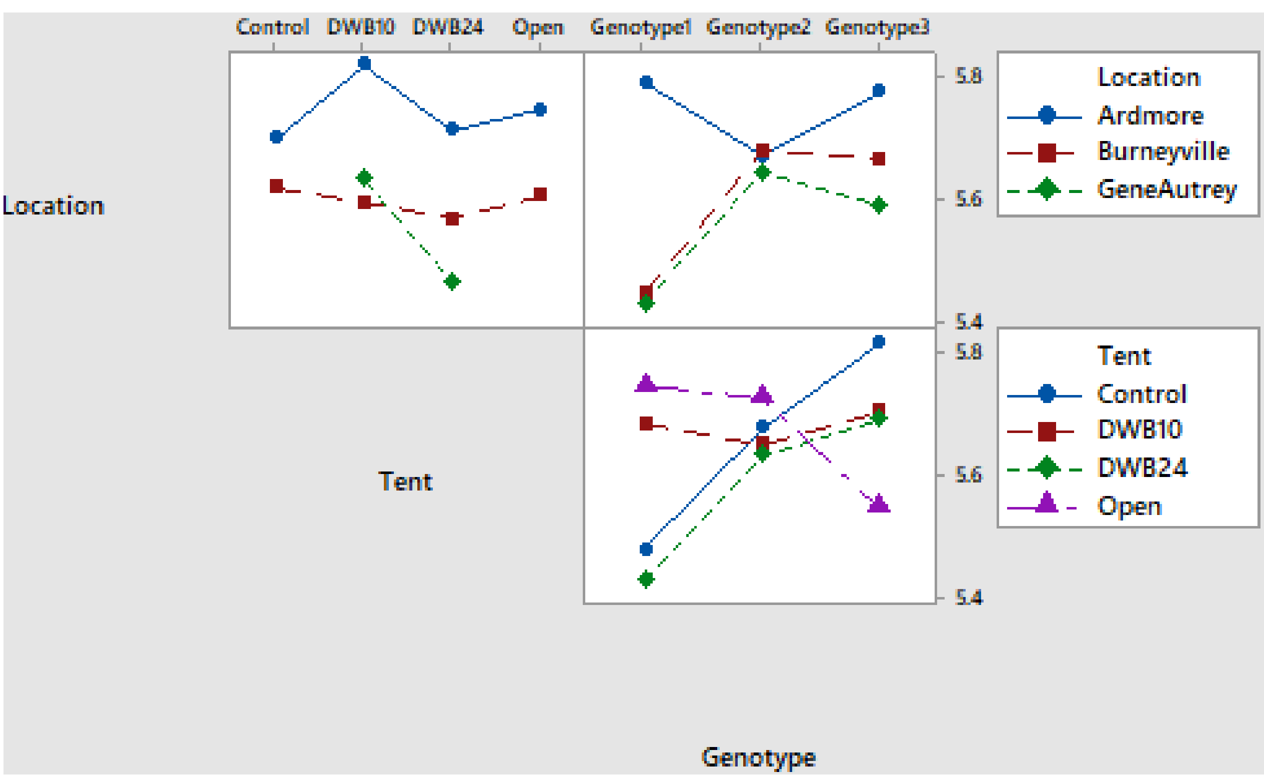

Figure 5. Plot for locations vs. PCT types and PCT type vs. genotypes for growth habit in 2019 trials.

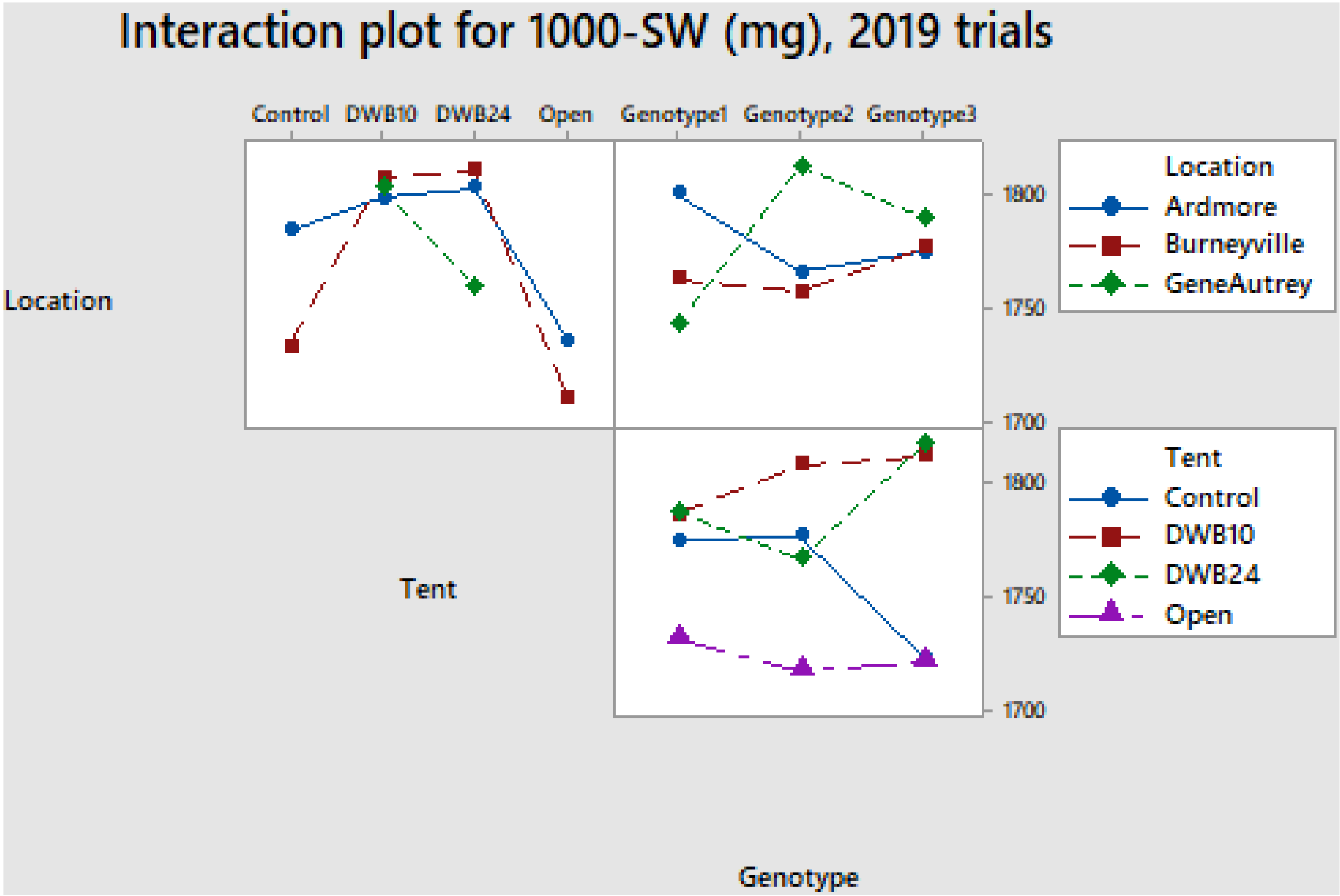

Figure 6. Plot for locations vs. PCT types and PCT type vs. genotypes for 1000-seed weight (mg) in 2019 trials.

Table 6. Mean squares (MS) from analysis of variance and their corresponding sum of squares (SS) as per cent of the total SS (in parentheses) for various traits in small pollination control tent (PCT) trials in 2018.

Source	df	PH	GH	SY	SW
Location	2	11.47 (2.1)	0.45 (4.8)*	8.40 (0.6)	6.96 (1.0)
Treatment	2	88.11 (16.4)**	0.15 (1.6)	1139.34 (78.0)**	111.17 (15.4)**
Genotype	2	2.85 (0.5)	0.47 (5.0)*	1.28 (0.1)	32.95 (4.6)*
Loc × Treat	4	5.99 (2.3)	0.21 (4.5)	3.17 (0.4)	10.38 (2.9)
Loc × Geno	4	6.20 (2.3)	0.10 (2.1)	8.36 (1.2)	15.64 (4.3)
Treat × Geno	4	7.76 (2.9)	0.08 (1.7)	17.40 (2.4)**	11.77 (3.3)
Loc × Treat × Gen	8	5.03 (3.8)	0.27 (11.4)*	11.10 (3.0)	17.21 (9.5)*
Error	108	6.92 (69.7)	0.12 (68.9)	3.86 (14.3)	7.94 (59.2)
Total	134				

*P<0.05, **P<0.01. Traits with non-significant mean squares not shown were: Disease Score, Ergot (%), and Seed Quality Score. PH= plant height (cm), GH= Growth habit (1-9; 1= decumbent, 2= semi-erect, 9= fully erect), SY= seed yield per plant (g), SW= 1000-seed weight (mg).

SW (Table 9).

Of more interest are the significant differences between treatments where the control had the highest performance for PH, SY and SW in 2018 and the DWB10 and DWB24 PCT treatments being numerically equal (Table 8). Interestingly, in 2019, control and open-control

Table 7. Mean squares (MS) from analysis of variance and their corresponding sum of squares (SS) as per cent of the total SS (in parentheses) for various traits in small pollination control tent (PCT) trials in 2019.

Source	df	PH	GH	SY	SW
Location	2	358.2 (26.9)**	0.47 (10.5)**	384.41 (21.6)**	8.78 (3.0)*
Treatment	3	112.9 (12.7)**	0.07 (2.4)	170.48 (14.4)**	47.65 (24.2)**
Genotype	2	4.8 (0.4)	0.18 (4.0)*	0.05 (0.0)	2.27 (0.8)
Loc×Geno	4	7.4 (1.1)	0.20 (8.8)**	19.99 (2.3)	11.65 (7.9)**
Treat×Geno	6	7.3 (1.7)	0.19 (12.5)**	15.05 (2.5)	8.30 (8.4)**
Error	132	10.4 (51.5)	0.04 (61.7)	15.14 (56.2)	2.63 (58.8)
Total	149				

*P<0.05, **P<0.01. Traits with non-significant mean squares not shown were: Disease Score (1 to 5; 0= no disease, 5=death due to disease), Ergot (%) and Seed Quality Score (1-5; 1= excellent, 5= extremely poor). PH= plant height (cm), GH= Growth habit (1-9; 1= decumbent, 2= semi-erect, 9= fully erect), SY= seed yield per plant (g), SW= 1000-seed weight (mg).

Table 8. Fitted mean values for main effects with significant mean squares in the analysis of variance for small pollination control tent (PCT) trials in 2018.

Factor	Detail	PH	GH	SY	SW
	Ardmore	-	5.89^{A}	-	-
	Burneyville	-	5.70^{B}	-	-
Location	Gene Autry	-	5.74^{B}	-	-
	SE m (±)	-	0.05	-	-
	LSD 5%	-	0.16	-	-
	DWB24	117.26^{B}	-	8.36^{B}	1813.4^{B}
	DWB10	117.31^{B}	-	7.83^{B}	1826.3^{B}
Treatment	Control	119.71^{A}	-	16.80^{A}	1905.2^{A}
	SE m (±)	0.39	-	0.29	13.30
	LSD 5%	1.28	-	-	43.64
	Genotype 1	-	5.89^{A}	-	1827.9^{B}
	Genotype 2	-	5.75^{AB}	-	1879.0^{A}
Genotype	Genotype 3	-	5.69^{B}	-	1838.0^{B}
	SE m (±)	-	0.05	-	13.30
	LSD	-	0.16	-	43.64

PH= Plant height (cm), GH= Growth habit (1-9; 1= decumbent, 2= semi-erect, 9= fully erect), SY= seed yield per plant (g), SW= 1000-seed weight (mg). Means that do not share a letter are significantly different.

showed significantly lower mean performance for PH, SY and SW than the two PCT treatments, DWB10 and DWB24, which were higher than controls but statistically the same (Table 9). Genotype 1 showed the highest performance in 2018 for GH but was the lowest in 2019 while genotypes 2 and 3 were average in both years. The SW mean of genotype 2 was significantly higher than other two genotypes in 2018 (Table 8).

Large PCTs-quantitative traits

Analyses of variance were performed separately for the two sites since they had different sizes and types of fabrics of large PCTs in 2019 (Table 10). Both PCTs had significantly higher SY and SW than their respective controls.

The trial at Unit 3 Farm showed significant mean squares for SY and SW with significantly higher mean values for the DWB10 PCT. The trial at HQ Farm also showed significant differences for SY, SW; the mean values for DWB24 PCT were significantly higher for SY and SW than the control at the same site. Comparison of DS, Ergot (%) and SQ here were significantly more favourable inside the DWB24 PCT than outside (lower scores) (Table 10).

Table 9. Fitted mean values ± standard errors for main effects with significant mean squares in the analysis of variance for small pollination control tent (PCT) trials in 2019.

Factor	Detail	PH	GH	SY	SW
Location	Ardmore	113.4±0.42^{B}	5.75±0.03^{A}	15.20±0.50^{C}	1780.3±6.6^{A}
	Burneyville	118.2±0.42^{A}	5.60±0.03^{B}	20.26±0.50^{A}	1765.4±6.6^{AB}
	Gene Autry	117.0±0.66^{A}	5.55±0.04^{B}	17.84±0.79^{B}	1749.5±10.5^{B}
Treatment	DWB24	118.1±0.48^{A}	-	19.13±0.58^{A}	1791.0±7.6^{A}
	DWB10	117.6±0.48^{A}	-	20.04±0.58^{A}	1802.8±7.6^{A}
	Control	115.1±0.64^{B}	-	14.69±0.77^{C}	1750.9±10.1^{B}
	Open	114.1±0.64^{B}	-	17.20±0.77^{B}	1715.7±10.1^{C}
Genotype	Geno 1	-	5.55±0.03^{B}	-	-
	Geno 2	-	5.67±0.03^{A}	-	-
	Geno 3	-	5.67±0.03^{A}	-	-

PH= Plant height (cm), GH= Growth habit (1-9; 1= decumbent, 2= semi-erect, 9= fully erect), SY= seed yield per plant (g), SW= 1000-seed weight (mg). Means that do not share a letter are significantly different.

Table 10. Mean squares from analysis of variance (above) and fitted mean values for various traits in large pollination control tent (PCT) trials at Unit 3 Farm and HQ Farm at Ardmore Noble Research Institute campus during 2019.

Farm	Source	df	SY	DS	SW	Ergot	SQ
Unit 3 Farm 6×6 tent (DWB10)	Treatment	1	119.81*	0.20	98701**	2.81	0.61
	Error	78	23.44	0.73	7571	2.11	0.29
HQ Farm 3×12 tent (DWB24)	Treatment	1	262.09**	0.61*	109520**	2.81+	3.20**
	Error	78	27.94	0.15	5789	0.89	0.32
Mean value							
Unit 3 Farm 6×6 tent (DWB10)	Tent	-	32.69	-	1853.8	-	-
	Control	-	30.25	-	1783.5	-	-
	SE m ±	-	0.77	-	13.8	-	-
HQ Farm 3×12 tent (DWB24)	Tent	-	31.96	0.03	1836.8	0.00	1.03
	Control	-	28.34	0.20	1762.8	0.38	1.43
	SE m ±	-	0.84	0.06	12.0	0.15	0.09

*P<0.05, **P<0.01, +P<0.08. SY= seed yield per plant (g), Disease Score (1 to 5; 0= no disease, 5=death due to disease), SW= 1000-seed weight (mg), Ergot (%) and Seed Quality Score (1-5; 1= excellent, 5= extremely poor). Mean squares for PH= plant height (cm), GH= Growth habit (1-9; 1= decumbent, 2= semi-erect, 9= fully erect) were non-significant and are not reported.

Germination percent

Small PCTs-germination percent

The analysis of variance showed significant location effect on germination at 7 and 14 days in both years but also at 21 days in 2018 only (Table 11). The treatment effect was only significant at 7 days in 2018 (Table 11). Mean germination percent was significantly higher at Ardmore in 2018 for each time point. In 2019, the germination percent at 7 and 14 days was higher at Gene Autry (Table 11). Mean germination of treatments were significantly different only in 2018 at 7 days. The mean germination of seed produced under the DWB10 PCT fabric was significantly higher than seed harvested under DWB24 fabric and control which were both similar at 7 days in 2018 (Table 11).

Despite the effect of locations on seed development and subsequently on rate of germination, the final germination percent on the 21st day was the highest at

Table 11. Mean germination (%) for main effects at 7, 14 and 21 days after sowing in small pollination control tent (PCT) trials in 2018 and 2019.

Factor	Loc/Treat	Means for small tents 2018			Means for small tents 2019		
		7 day	14 day	21 day	7 day	14 day	21 day
Location	Ardmore	12.89^{A}	74.67^{A}	94.22^{A}	14.22^{A}	72.67^{B}	93.17^{A}
	Burneyville	10.67^{B}	66.00^{C}	89.33^{B}	12.30^{B}	71.17^{B}	91.67^{A}
	Gene Autry	11.56^{AB}	70.44^{B}	92.67^{A}	14.83^{A}	75.92^{A}	93.75^{A}
	SE m (±)	0.57	1.09	0.78	0.51	1.28	1.39
	LSD 5%	1.69	3.24	2.32	1.48	3.73	4.07
	Significance	*	**	**	**	**	NS
Treatment	DWB24	10.67^{B}	-	-	-	-	-
	DWB10	12.89^{A}	-	-	-	-	-
	Control	11.56^{B}	-	-	-	-	-
	SE m (±)	0.57	-	-	-	-	-
	LSD 5%	1.69	-	-	-	-	-
	Significance	*	-	-	-	-	-

*P<0.05; **P<0.01; NS= Not significant. The ANOVA (not given) had locations (2 df), treatments (2df) and error (18 df) since locations × treatment interactions were not significant in any case. Means that do not share a letter are significantly different. Means for non-significant treatments are not given.

all locations in both years (Table 11). The lowest overall germination of 89% was recorded for Burneyville in 2018. The significant difference between locations and for genotypes tends to disappear as the time from sowing seeds increased. The slower start of germination in some cases may be due to the effect of climate at different locations for the stored metabolites to be activated differentially.

Large tents-germination percent

There was no significant variation between treatments for germination percent at 7, 14 and 21 days following sowing. There was a linear increase in the percent of germinated seeds from 7 to 21 days and the germination reached more than 96% for seed from both farms and PCT types. At 7, 14 and 21 days DWB10 PCTs showed 12, 70 and 97% germination respectively, against 12, 70 and 96% for the control. Seeds from the PCT DWB24 showed 13, 66 and 97% germination at 7, 14, and 21 days vs. 14, 72 and 97% for the respective control. Since there was no significant difference in germination of seed from the larger PCTs or from the outside control, it can be concluded that the PCT microenvironment from either fabric in no way differed in its effect on the rate of seed germination or viability of seeds.

Fan effect-small tents

Fans were introduced in tents at Ardmore and Gene Autry, but not at Burneyville during 2019 trials on small tents. Fan vs. no fan effects were significant for PH, GH, SY and for germination percent at 7 and 14 days (Table 12). Seeds produced with fans in the PCTs and control always gave higher mean percent germination at all days of the count. Provision of fans tended to produce plants with lower PH, higher GH score and lower SY without affecting the seed size. Higher SY may not mean higher germination since healthy and viable seeds may be fewer than the actuals. Fans could have created a microclimate that produced seeds, which looked similar in weight to those under no fan, but had better metabolite reserves resulting in better germination that might translate in better establishment and stand in the field. Further, reduction in SY by fan airflow might be caused by pollen mobility to be adversely affected reducing settlement on stigmas. Thus, there is no apparent advantage of adding fans in the PCTs. Fans × treatment interactions were significant for PH and SY (Table 12 and Figure 7). The major source of interaction was the interaction of two types of tents with fans in them. The PH of DWB10 was reduced in the presence of a fan but SY increased in comparison with the DWB24 PCT. Perhaps conditions in the heavier and waxier fabric of DWB10 improved seed set and SY compared to the more aerated DWB24 PCT material (Figure 7).

DISCUSSION

The major objective of this study was to assess the comparative performance of grass genotypes in novel nonwoven synthetic fabric PCTs vs. isolated, open pollinated control conditions at different locations in

Table 12. Mean squares from analysis of variance (above) and mean values (below) for fan effect on quantitative traits and germination (%) at 7, 14 and 21 days after sowing in small pollination control tent (PCT) trials in 2019.

Source	Df	PH	GH	SY	Df	7 day	14 day	21 day
				Anova				
Treatment	3	168.91**	0.07	226.28**	3	2.32	4.02	5.41
Fan vs. no fan	1	621.72**	0.31*	721.29**	1	29.76*	45.76*	23.05
Treat × Fan	3	154.35**	0.03	77.11**	3	1.43	5.50	3.37
Error	142	8.44	0.06	14.61	22	4.76	8.03	8.06
Total	149	-	-	-	29	-	-	-
				Mean values				
No Fan	-	118.20±0.38	5.60±0.03	20.26±0.49		12.33±0.63	71.17±0.82	91.67±0.82
With Fan	-	113.94±0.33	5.69±0.03	15.68±0.43		14.42±0.55	73.75±0.71	93.50±0.71
% increase/decrease over no fan	-	-3.60%	1.70%	-22.63%		16.90%	3.63%	2.00%

*P<0.05, **P<0.01. PH= plant height (cm), GH= Growth habit (1-9 scale), SY= seed yield per plant (g).

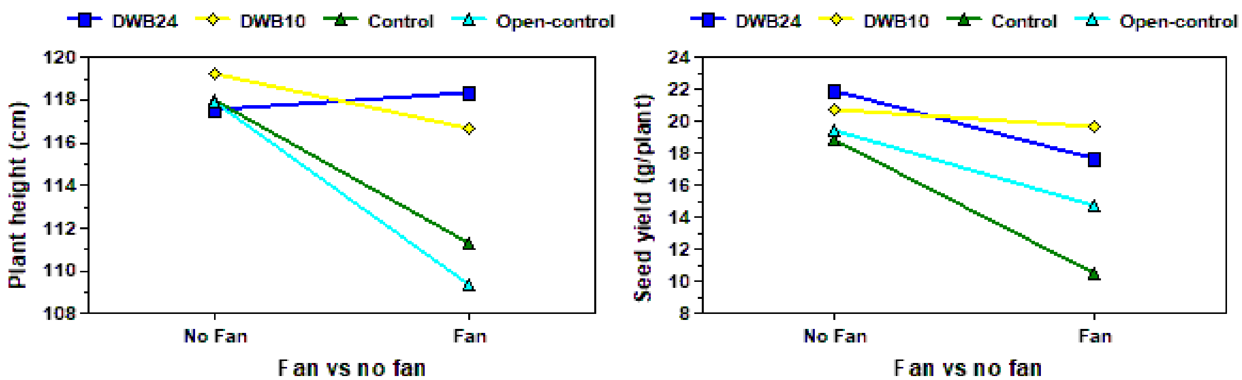

Figure 7. Interaction plots for fan effect vs treatments for plant height (cm) and seed yield (g/plant) in small pollination control tents (PCT) in 2019 trials.

Oklahoma. Locational differences were more pronounced in 2019 with significant differences for all quantitative traits when differences only existed for SW and GH in 2018. The treatment differences were consistently significant for most of the traits across years, which revealed possibilities of more productive options over open pollinated controls. There also existed significant interactions of treatments with locations and genotypes for SY and SW in two years, but the contribution of interaction sum of squares (SS) to the total SS was very small reaching a maximum of 13% for GH in 2019 (Tables 6 and 7). These contributions were very small in comparison with the larger contribution of the main effects to the total SS. Therefore minimal significance was attributed to these interactions and conclusions were based largely on main effects (Tables 6 and 7).

Of the two years, 36 to 87% more seed per plant was produced in 2019 across sites compared to 2018. The two PCTs showed a 2 to 5% decrease for PH, SY and SW compared with the control treatment in 2018 (Figure 8). However, in 2019 the performance of tall fescue was superior to control for PH, SY, SW in small PCTs, and SY and SW in large PCTs. SY from DWB10, DWB24 small PCTs were 37 and 30% higher, respectively, over the control (Figure 8). Similarly, SY from large DWB24 PCTs were 13% higher and the DWB10 PCTs averaged 8% higher over the control (Figure 8). Clearly, SY returns from both PCTs were higher than open controls (Tables 8 and 9); thus both PCT materials were equally useful in this particular climate and crop combination. However, the choice of PCT fabric for other crops and other climates may be different.

Viable pollen is important for species dispersal, fitness, and survival of the next plant generation (Impe et al., 2020). It is also essential for directed plant breeding and, consequently, crop improvement. The extent of seed set following pollination, fertilization and healthy seed development is conditioned by the ambient microclimate within the PCTs. Wang et al. (2004) assessed *in vitro* pollen viability from transgenic and non-transgenic tall

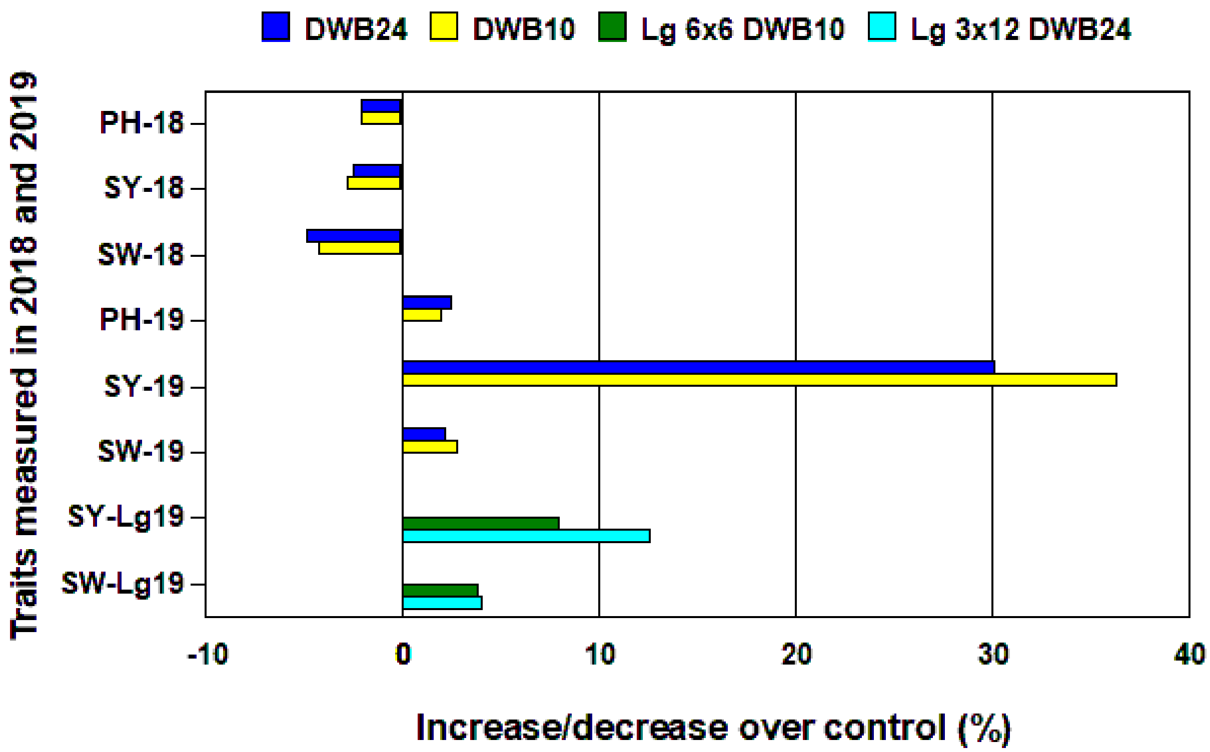

Figure 8. Percent increase or decrease of mean performance of various quantitative traits over control for small tents DWB10 and DWB24 in 2018 (with -18) and 2019 (with -19) and large tent (Lg) for 2019. PH= plant height, SY= seed yield, SW= 1000-seed weight.

fescue and found that treatment with relatively high temperatures (36 and 40°C) reduced pollen viability while relative humidity did not significantly influence pollen viability. They found that the viability of pollen from transgenic progenies was similar to that from seed-derived control plants. Plant disease can also decrease seed production, especially in tall fescue (Barker et al., 2003). In the Pacific Northwest of the USA, the most significant diseases affecting seed production of tall fescue are fungal diseases, including stem rust, caused by *Puccinia graminis* subsp. *graminicola* Pers., and blind seed, caused by *Gloeotinia temulenta* (Prill & Declacr.) (Alderman et al., 2009). However, the appearance of plant disease is highly influenced by environmental factors (Velásquez et al., 2018). Even when a host is susceptible, the plant may not be infected by a virulent pathogen if the environmental conditions are not optimal for disease. Therefore, the occurrence of diseases within plants and the developing seed in tent microenvironments is highly influenced by the inside temperature and relative humidity-the two major contributing factors. The appropriate humidity ensures leaves remain moist and the temperature ensures warmth for germinating spores of disease pathogens. In general, there was no difference in temperature and relative humidity between the two PCT fabrics across all locations and years. While the lower temperatures in the PCTs fell below the control by a few degrees the maximum and average temperatures were higher than outside. Similarly, the minimum humidity was generally lower in PCTs in both years and across all locations, but the maximum humidity was the same or higher in PCTs than outside. The average humidity was equal or lower in the PCTs vs. the outside groups. Moderate temperature and relative humidity in PCTs tended to favour higher SY and SW with disease free seeds of better quality (Table 10).

The effect of introducing fans within PCTs and controls to determine if they improved pollination, fertilization and subsequent seed output were also examined. We hypothesized that static air within the PCTs might reduce the free airflow of pollen grains leading to poor seed set. However, the introduction of fans unexpectedly decreased seed yield by 23% (Figure 9). While no explanation could be evidenced, it is possible that the draft created by the fans could have interfered with the settling of pollen on receptive stigmas during pollination and fertilization However, the reduction of SY with fans established that there is no need for increased airflow within the PCTs; perhaps the porous nature of the fabrics allowed enough aeration and airflow within the PCTs eliminating the need for increasing it by other means.

A measure of healthy seed development is germination capability (McDonald and Copeland, 1997) which

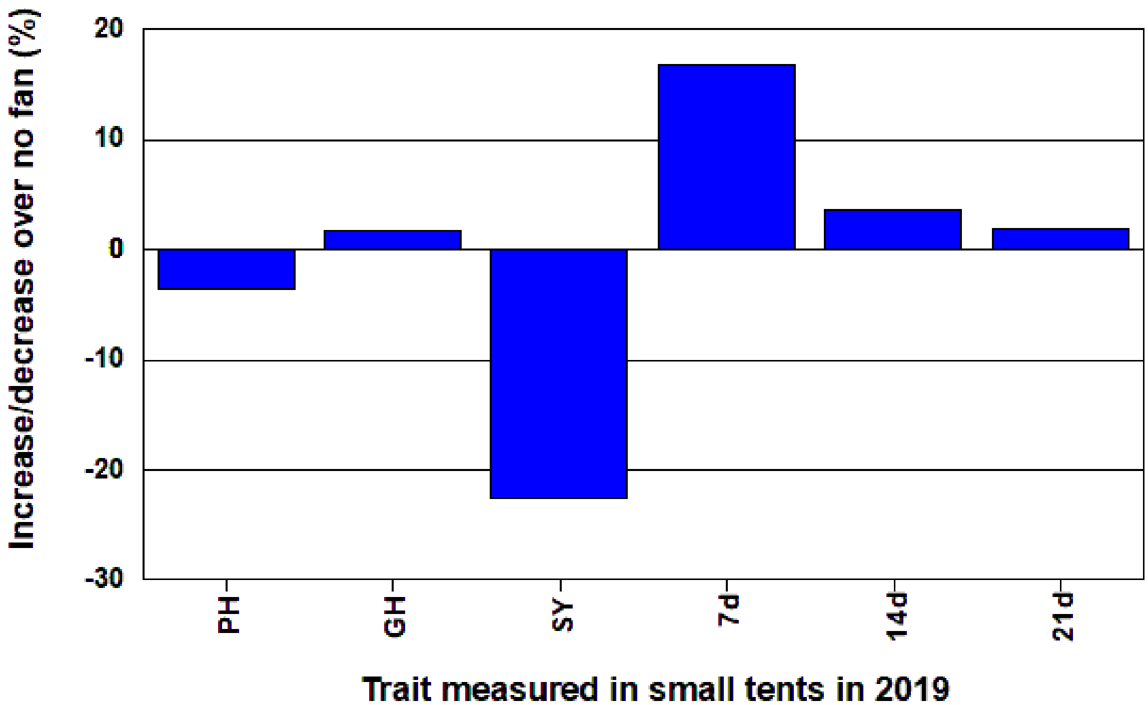

Figure 9. Fan effect as (percentage) increase or decrease of mean performance of various quantitative traits and germination (%) after 7, 14 and 21 days over no fan in small pollination control tents (PCTs) 2019 trials. PH= plant height, GH = growth habit, SY= seed yield.

indicates not only seed viability but also the extent of the store of differential metabolites responsible for faster or slower germination. There was evidence of significant differences for germination rate at all stages of count for different locations with Burneyville seed displaying average germination rates and the other two locations changing their ranks at some stages. This can be expected as seed produced at different locations can differ in quality and extent based on stored metabolites. However, one would not expect differences among seed produced in PCTs and outside controls if, conditions within PCTs are ambient. In general, germination of seed produced in the two PCTs and outside controls were comparable at all stages except at 7 days when seed produced within PCTs with the DWB24 fabric had higher germination. Overall, there was little difference among treatments and locations for the final seed germination at 21 days after sowing. This demonstrates that seed produced in PCTs exhibit similar germination to the seed produced under natural conditions and that the use of a PCT for seed multiplication could be a gainful possibility.

An important feature of hybridization or seed multiplication in PCTs is the maintenance of genetic identity of stocks from contamination of foreign or unwanted pollen. We did not have any evidence, though preliminary, for any contamination from outside pollen in the PCTs. This is a very useful indication to build the confidence of plant breeders and seed producers for the use of nonwoven fabric PCT's in grass breeding.

Economic implications

While a proper economic analysis was not a direct objective of the present research, we can examine the effect of various factors determining the economic impact of using PCTs in comparison with other means of isolation or self-pollination. This is a very preliminary analysis that could be used as a basis for future studies and follows a simplistic approach shown in Schaffert et al. (2016) and Gaddameedi et al. (2017) in sorghum. Our approach is based on explorative circumstantial evidence from the analyses provided by the available data that could be extrapolated for comparative assessments (Table 13).

While performing any economic analysis for grass breeding it should be remembered that a grass breeder is interested in: (i) attempting single or multiple interspecific crosses, (ii) seed increase of interspecific crosses for synthetic varieties, (iii) seed increase of advanced entries for multi-locational trials, (iv) maintenance of early generation of seed such as nucleus or breeder seed, and (v) maintenance of genetic stocks for use in breeding. While making interspecific crosses, objectives are usually identifying good combining components for synthetics or identifying specific cross combinations for releasing hybrid varieties for their increased heterosis. Interspecific single crosses between two species are made by hand using pollination control bags to get small quantity of seeds. However, for multiple crosses (e.g., several

Table 13. Factors for comparing pollination control tents (PCTs) for economic analysis.

Treatment	Seed yield	Diseases	Effect of natural factors	Bird damage	Labour, resources	Risk of loss of genetic stock	Reusability	Relative cost†
PCT	= or > control; >30% vs control for small tent sand 8-13% > for large tents	Variable	Wind, rain, storm effects small	Nil	Low, 30-40 mts for 3 people to assemble, and to remove	Nil	Yes	$$
Isolation plots	=control	Diseases occur, ergot	High impact	Can be high	High; 3 h of 1 person per week for full season	Low with costly watch and ward. Part or whole loss from uncontrolled animals.	NA	$$$
Isolation chambers	< control	Variable	Nil but expensive climate control	Nil	Permanent type, high cost of temperature, humidity, lighting etc.	Nil	Yes	$$$
Bagging	<, plant × plant crosses only	Variable	Wind, rain may tear or blow away	Variable	Only for bagging or re-bagging	Nil	Paper not; synthetic yes	$
No bagging	=control	As in isolation	High effect	Can be high	Nil	As in isolation	NA	Nil

†The dollar ($) sign indicates relative costing for each method. The method with one $ has minimum cost, $$ has double and $$$ has three times more price.

female parents crossed with one good combining male parent) or for seed increase, space isolation plots, isolation chambers or PCTs will be appropriate. In all of these scenarios, breeders place a high level of confidence in the genetic integrity, quality and viability of the seed produced.

Traditionally, plant breeders used pollination control bags made of paper, but recently synthetic fabrics with greater strength against bad weather, bird damage and wind resistance along with air permeability, lower moisture absorption and prevention of unwanted pollen have been developed (PBS Intl., 2020a,b). Pollination control bags made from nonwoven synthetic fabrics have been successfully trialed and proven to deliver better outputs and increased plant breeding efficacy than controls by Gitz et al. (2015), Schaffert et al. (2016, 2018, 2019) and Gaddameedi et al. (2017) in sorghum; Clifton-Brown et al. (2018) in sugar beet, wheat, *Arabidopsis* and *Miscanthus*; Hayes and Virk (2016) in *Miscanthus*; Vogel et al. (2014) and Adhikari et al. (2015) in grasses; and Bonneau et al. (2017) in oil palm. Encouraged with the superior performance and re-usability of nonwoven synthetic fabrics for pollination control bags we uniquely extended the use of such fabrics to PCTs in the present study with the objective of improving the efficiency of grass breeding and seed production.

Hayes and Virk (2016) compared the efficiency of isolation chambers (small pollen-proof compartments with controlled airflow and water supply) with pollination control tents in both external and glasshouse environments in *Miscanthus*. The comparative efficiency of tents and isolation chambers was measured by recording the total number of seeds and average number of seeds per head, which were both consistently higher for tents whether in external or internal glasshouse conditions. Thus the synthetic nonwoven polyester fabric of the tents, as used in the present study, provided an ambient climate for higher seed set. The temperature and humidity inside the crossing tent followed the same pattern as shown by the ambient conditions in the Venlo glasshouse. The temperature and humidity in the glasshouse isolation chamber was lower than both the crossing tent and the ambient conditions of the Venlo glasshouse. The difference in humidity and temperature within the different crossing environments was likely the reason there was reduced seed set, on average, between the isolation chambers when compared with the results from the crossing tents.

The seed yield, over a 15-year average, for a tall fescue plant in the breeding program at the Noble Research Institute ranges from 20.00 to 26.50 g. This means seed yields of 20 g/plant or higher would justify the use of PCTs or isolation chambers for seed increase on a regular basis. While 2018 was not a good year for seed yields, being much lower than expected, the yields in 2019 in smaller PCTs were closer to 20 g per plant and higher than in the outside control.

This showed that the use of PCTs could be an economic possibility for seed increases in grasses in at least Oklahoma climate conditions.

In 2018, the first small PCT took about 1.5 h (3 people) to assemble. This included digging and anchoring of the skirting and placing a soaker hose for irrigation of the plants under the PCT. In 2019, it averaged about 30 to 40 min per small PCT for three people to complete the task. However, more labour is required with the control isolation plots from a maintenance standpoint. Maintenance around the isolation controls usually requires planting a pollen screen of cereal rye (*Secale cereale* L.) as well as hoeing and/or spraying to reduce weeds or insect pests. In addition, there is the added issue of maintaining the land around the isolation plot. Experience in Oklahoma shows that one full time person spends about 3 h per week working on keeping the outside control nursery clean of weeds or insect pests. About 0.5 h per week (1 employee) were spent on maintenance of each of the large PCTs. However, it could be possible to raise revenue through the sale of the grain produced by the pollen screen (cereal rye) or potentially other types of crops, to offset the cost of maintaining open type nurseries.

With the open pollinated controls, the only way to maintain genetic purity is with distance or isolation from the same species. For open pollinated species, such as tall fescue, a minimum of 305 m of distance between seed fields is required for the production of breeder or foundation certified seed as recommended by the Oregon Seed Certification Service (Oklahoma Crop Improvement Association standards are the same). If a breeding program established 20 or more open pollinated seed increases each year, the distance requirements would be demanding, requiring a spread of isolation nurseries over lots of different farms at different locations creating administrative and logistical challenges. If land space is a factor, then the number of isolations planted could be an issue, which may cost a generation of advancement. PCTs with reliable seed production would allow the planting of many isolation plots in a much smaller area. This would reduce time for traveling to and from many different locations and maintaining the space around these locations. For this purpose, researchers may prefer the larger PCTs compared to the smaller ones. Since the small PCTs are portable and easy to move they could also be used at leased offsite locations, such as private agricultural producers and universities. The PCTs would be much easier to maintain at these types of locations vs. larger open pollinated plots since travel to these sites may be many kilometres away. PCTs may reduce costs since less time is spent at the location for nursery maintenance. In addition, the production of high-grade seed or breeder (nucleus) seed of a licensed cultivar normally costs a seed company around \$35 to 50 kg^{-1} to produce. In this scenario, pre-breeder seed would be a good target for the small PCTs, while the large PCTs would be ideal for breeder or nucleus seed production.

Since our experiments, a number of modifications for improvement of PCTs have been made. Previously the seams of the cover in the corner of the roof tended to show some wear and tear. This has been improved with the new robust frame structures and methods of fixing the cover fabric. It was also felt that some type of ‘U’ type anchor for holding the frame on ground could have been useful. This improved design is more robust and holds on the ground much more strongly than in previous versions. Options for windows are provided in the new design that allows viewing the interior of the PCT without disturbing it.

Future considerations

Although tents have been used for indoor and outdoor plant multiplications, the use of specifically developed PCTs as pollination control aids and seed increases are recent. Therefore, there is a market for the development of robust structures that can withstand high winds and bad weather, but are lightweight for transport, easy to assemble and include windows for examination and easy entry. Improvement regarding irrigation and agronomic operations within the PCTs without disturbance were needed following our experiments. Advances have been made since these trials and the PCT design has been improved to increase the benefit: cost ratio and for wider applicability to many crops. Flexibility in sizing the PCT covered area is also important for accustoming the protected area as per breeders’ requirement in any season. Advances in this area reflect development of PCTs of specific capacity that can be joined together as a modular structure to a number of independent parts to cover as large an area as required.

The second important aspect is the use of the right fabric as a cover. The fabric needs to be easily fitted, but hard enough to withstand wear and tear on the corners where it touches the frame, be pollen proof, but have sufficient aeration for temperature and humidity control. Apart from the DWB10 and DWB24 synthetic nonwoven fabrics used in the study, there are a number of other fabrics that are available that have been tested in other crops such as sugar beet (Paul Townson Pers. Comm.) and mustard (S.S. Banga Pers. Comm.) with encouraging results. However, while the present study has established the superiority of synthetic PCTs, further studies to confirm wider utility in other crops and breeding scenarios will be needed in terms of estimating the economic implications in seed production.

Conclusions

Pollination control tents (PCTs) made from two nonwoven synthetic fabrics, DWB10 and DWB24, were tested against open controls across two years and three locations in Oklahoma for their seed production efficacies and control of pollen contamination. The two types of PCTs showed similar and higher seed yield by up to 36% compared with open control treatment. The higher average temperature and a lower to average humidity within the PCTs compared to the control across locations and years could have led to the more optimal and healthier seed set in the PCTs. The introduction of fans in the PCTs to increase pollen flow was not beneficial as it reduced seed yield by about 23% demonstrating that natural conditions in the PCTs were conducive for higher seed yield. Knowledge gained from this study is being used to improve the PCT design structure and to test newly developed fabrics in different crops. The proposed economic analysis and the generalized possibilities regarding the application of PCT technologies in plant breeding and in particular grass breeding, seems encouraging for increasing seed outputs, the hybridization process and seed multiplications.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Adhikari L, Anderson MP, Klatt A, Wu Y (2015). Testing the efficacy of a polyester bagging method for selfing switchgrass. BioEnergy Research 8:380-387.

Alderman SC, Pfender W, Ocamb CM (2009). Diseases in Seed Production. In Tall Fescue for the Twenty-first Century (eds H. Fribourg, D. Hannaway and C. West). doi:10.2134/agronmonogr53.c24

Barker RE, Pfender WF, Welty RE (2003). Selection for Stem Rust Resistance in Tall Fescue and Its Correlated Response with Seed Yield. Crop Science 43:75-79. doi: https://doi.org/10.2135/cropsci2003

Brown J, Caligari P, Campos H (2014). Plant Breeding, 2nd edition. Wiley Blackwell, West Sussex, UK p 271.

Bonneau L, Eli D, Vovola P, Virk DS (2017). Comparing pollination bag types for micro-environmental parameters influencing seed production in oil palm. Journal of Oil Palm Research 29(2):168-179.

Clifton-Brown JC, Senior H, Purdy SJ, Horsnell R, Lankamp B, MuEennekhoff A K, Virk D, Guillemois E, Chetty V, Cookson A, Girdwood S, Clifton-Brown G, Tan ML, Awty-Carroll D, Bentley AR (2018). Investigating the potential of novel nonwoven fabrics for efficient pollination control in plant breeding. PLoS ONE 13(9):1-21.

Darbyshire SJ, Pavlick LE (2012). Festuca. Archived 2012-10-25 at the Wayback Machine Grass Manual. Flora of North America.

Erdtman G (1943). An Introduction to Pollen Analysis. Chronica Botanica Company, Waltham, 1-239.

Gaddameedi A, Kumar AA, Madhavrao PR, Virk DS, Senior H (2017). Evaluating the efficacy of synthetic fibre pollination control bags in Sorghum during the rainy season. International Journal of Plant Breeding and Genetics 11(1):39-54.

Geisler F (1945). A pollen study of thirty-two species of grasses. In Ray C. Freisner (Ed.). Butler University Botanical Studies (1929-1964) 7(6):65-73. Retrieved from: https://digitalcommons.butler.edu/botanical/vol7/iss1/6

Gitz DC, Baker JT, Xin Z, Burke JJ, Lascano RJ (2015). The microenvironment within and pollen transmission through polyethylene sorghum pollination bags. American Journal of Plant Science 6:265-274.

Hayes C, Virk DS (2016). Assessing the relative efficacy of polyester pollination bags and crossing tents, and isolation chambers for seed harvest in *Miscanthus* crosses. International Journal of Plant Breeding and Genetics 10(2):79-90.

Impe D, Reitz J, Köpnick C, Rolletschek H, Börner A, Senula A, Nagel M (2020). Assessment of Pollen Viability for Wheat. Frontiers in Plant Science 10:1588.

McDonald MB, Copeland LO (*1997)*. Seed production: Principles and practices. Chapman and Hall, New York. doi:10.1007/978-1-4615-4074-8

Moore KJ, Moser LE, Vogel KP, Waller SS, Johnson BE, Pedersen JF (1991). Describing and Quantifying Growth Stages of Perennial Forage Grasses. Agronomy Journal 83:1073-1077. doi:10.2134/agronj1991.00021962008300060027x

Oregon Seed Certification Handbook (2018). Oregon Seed Certification Service. Oregon State University. https://seedcert.oregonstate.edu

PBS International (2020a). Intelligent design-why our bags are best. Retrieved on 20 April 2020. https://www.pbsinternational.com/our-products/intelligent-design/

PBS International (2020b). Grasses research-why our bags are best. Retrieved on 20 April 2020. https://www.pbsinternational.com/our-products/grasses-products/grasses-research/

Schaffert RE, Virk DS, Senior H (2016). Comparing pollination control bag types for sorghum seed harvest. Journal of Plant Breeding and Crop Science 8(8):126-137.

Schaffert RE, Virk DS, Senior H (2018). Are nonwoven synthetic pollination bags a better choice for sorghum breeding? Journal of Plant Breeding and Crop Science 10(3):58-68.

Schaffert RE, Virk DS, Senior H (2019). Are Nonwoven Synthetic Pollination Bags a Better Choice for Sorghum Breeding? In Current Research in Agriculture and Horticulture 1(Chapter 9):82-95. Print ISBN: 978-81-940613-7-3, eBook ISBN: 978-93-89246-27-8. DOI: 10.9734/bpi/crah/v1

Scott AG, White DWR (1988). Variation among perennial ryegrass x tall fescue plants from tissue culture. Proceedings of the New Zealand Grassland Association 49:81-86.

Sokal RR, Rohlf FJ (2011). Biometry: The Principles and Practice of Statistics in Biological Research. 4th Edn., W.H. Freeman and Co., New York, ISBN-13: 978-0-7167-8604-7, P. 937.

Velásquez AC, Castroverde CDM, He SY (2018). Plant and pathogen warfare under changing climate conditions. Current Biology 28(10):R619-R634. doi:10.1016/j.cub.2018.03.054.

Vogel KP, Gorz HJ, Haskins FA (1989). Breeding grasses for the future. In: D.A. Sleper, K.H. Asay, & J.F. Pedersen (Eds.), Contributions from Breeding Forage and Turf Grasses. Crop Science Special Publ. 15, pp. 105-122. Crop Science Society of America, Madison. WI.

Vogel KP, Sarath G, Mitchell RB (2014). Micromesh fabric pollination bags for switchgrass. Crop Science 54:1621-1623.

Wang ZY, Ge Y, Scott M, Spangenberg G (2004). Viability and longevity of pollen from transgenic and nontransgenic tall fescue (*Festuca arundinacea*) (Poaceae) plants. American Journal Botany 91(4): 523-530.

Wodehouse RP (1935). Pollen Grains. McGraw-Hili Book Co., Inc. pp. 303-320.