COMPARING POLLINATION BAG TYPES AND THE EFFECT OF ENVIRONMENTAL CONDITIONS ON SEED SET IN *MISCANTHUS* CROSSES

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Three bag types, i.e., PBS International (**dura**web®), a Paper-&-Plastic bag, and Glassine were compared for their relative efficiency in producing a successful cross in *Miscanthus* under two environments of glasshouse and isolation chamber conditions. Crosses were attempted in 2013 at the Institute of Biological Environmental and Rural Sciences (IBERS), Aberystwyth University.

ANOVA: The effect of two climatic conditions (glasshouse and isolation chambers) was non-significant on seed set and success of crosses. Bag type and Type of cross, however, had significant effect on seed setting upon pollination. None of the interactions were significant.

Bag type effect: While the Glassine and Paper-&-Plastic types did not differ significantly, the PBS International bags were superior to the combined effect of other two types of bags with 12% higher rate of success of crosses.

The success rate of crosses with PBS' **dura**web® bags was the highest at 45%, which exceeded Glassine by 15% and Paper-&-Plastic over 7%. The Z-test revealed highly significant higher mean rate of success for the **dura**web® bags than the combined mean success rate of Glassine and Paper-&-Plastic type of bags.

Cross type effect: The success rate of crosses was significantly higher when crosses involved genotypes of the same *Miscanthus* species than when they represented different species. The mean success of crosses was 47% from intraspecific crosses which was 13% higher than the interspecific crosses.

Seed traits: Seeds produced within PBS bags were significantly bolder (heavier) and presumably healthier for better seedling establishment under stress environments.

Economic analysis: Average cost of a cross using different bags was computed from the actual spend. The most economical bags are those of PBS International **dura**web® type which are cheaper by £55 pounds than glassine and £19 than Paper-&-Plastic type for a cross. The **dura**web® bags give relative gain of 33% over glassine and 15% over Paper-&-Plastic type. Considering many crosses attempted in a breeding programme our simulations showed that for 1000 crosses, the **dura**web® bags result in saving of £55,000 over Glassine and £19,000 over the Paper-&-Plastic type of bags. Even when 500 crosses are attempted the saving still remains very significant.

Reuse: The PBS International bags stood up well to the different climatic conditions and were easy to re-use with an autoclave cycle in between. The Paper-&-Plastic bags were unable to withstand an autoclave cycle and so for single use bags were too costly. They were also a bit too short for use with *Miscanthus* species. The glassine bags became brittle under the different climatic conditions and as such were only usable once.

Both of the glassine and the paper and plastic bags were damaged by slugs and were broken by the continued growth of the *Miscanthus* stems. These problems were not observed in the PBS bags.

In grasses, pollination takes place by wind. However, when controlled hybridisation is undertaken the flower heads are enclosed within a pollination bag to exclude extraneous pollen from contaminating. Pollination bags have a very crucial role in the success of hybridisation programme; they must be impermeable to pollen of any species capable of fertilizing the species concerned, and provide ambient environmental conditions within for healthy seed development following pollination and fertilization by the desired pollen. Therefore, the quality of bag does not only determine the rate of success of seed set but also ensures the genetic integrity of the cross.

Great strides have been made in synthetic mate rials that can be used for making pollination bags. Of the various materials with woven and pressed fibres some are capable of producing an idealcrosses were computed by dividing the num ber of crosses with seed set by total number of crosses attempted for a cross combination and multiplied environment within them for seed development. However, limited information is available on the comparative efficiency of different materials for their suitability for pollination bags that result in enhanced seed set following pollination. A good pollination bag should be durable, remain intact, prevent moisture collection, allow aeration but preventing pollen or insect invading the flowers, and be easy to be handled by the worker. Also how these bags interact with the outer environment or climatic conditions may determine their strength. The fibre of some bags is such that they can be re-used after treatment compared with others.

The objective of this study was to compare the efficiency of purpose-made bags with the most commonly used ones by grass breeders under glasshouse and isolation chamber conditions.

MATERIALS AND METHODS

A total of 562 pairwise interspecific and intraspecific crosses were made in *Miscanthus* during the 2013 crossing season at IBERS, Aberystwyth University, Wales, UK. The *Miscanthus* species used were: *M. sinensis*, *M. sacchariflorus*, *M. transmorrisonensis*, *M. floridulus*, *M. condensatus* and hybrids (these included naturally occurring hybrids and ones which were made during the course of the breeding programme).

The paired crosses were made using three types of pollination bags. There were: Glassine bags made of glazed paper, Orchard Wholesale crossing bags made from wet strength kraft paper and a plastic panel for visibility, and PBS International bags from PBS International made from a bonded polyester material known as **dura**web®, breathable and pollen proof. The glassine bags were disposed of after a single use as they were not reusable. However, PBS International and Paper-&-Plastic bags were autoclaved after use or re-using in the crossing programme. This worked well for the **dura**web® bags but the Paper-&-Plastic bags came apart after this treatment and as such were only suitable for single use.

Crosses were made under two climatic conditions; glasshouse and isolation chamber. Four modified compartments of a Venlo glasshouse were used for paired crosses. These compartments have temperature control through automatic roof vents, reflective screens and heating pipes, supplementary lighting and irrigation via capillary matting, drip feeders and overhead spray nozzles. These compartments were controlled with a Campbell logger in order to set the temperature and photoperiod climate required to induce flowering in a sub-tropical species under temperate conditions. The isolation chambers are small pollen-proof compartments which can be used to cross groups of plants in isolation from any external pollen. The air is filtered before being blown into all compartments which aids the distribution of pollen in the chamber and also maintains a positive air pressure in the compartment to prevent the entry of external pollen. The chambers were irrigated by capillary matting which was kept wet by means of a header tank.

Data were collected on percentage success of crosses. Paired combinations of genotypes that showed seed set in the bag following pollination were taken as successful cross.

During the course of the crossing season bags were also monitored for temperature and humidity within them and compared with the ambient conditions. Temperature in °C and humidity were recorded inside and outside the bags daily from the first week of May to July, by the use of a Tinytag Extra TGX-3580.

The seed set following pollination was taken as the success of a cross. Percentages of successful by 100. Data on percent successful crosses were analysed by performing an analysis of variance on percentages and their angular transformations since there occurred a number of crosses with 0 and 100% of success. Therefore percentages were transformed to Arcsin or angular transformation for proportions where angle = arcsin√ percentage. In the angular transformation proportions near zero are spread out so as to increase their variance. With n<50, a zero proportion is counted as 1/4n before transforming to angles, and a 100% proportion as (n-1/4)/n. In our case number of crosses per cross combination was always <50. However, the angular transformation does not remove inequalities in variance arising from different values of n.

The analysis of variance allowed for partitioning of the sum squares for bag types into the following orthogonal contrasts:

Comparison	Coefficient		
	duraweb ®	Paper-&-Plastic	Glassine
duraweb® Vs Others	2	-1	-1
Glassine Vs Paper-&-Plastic	0	1	-1

A Z-test was performed for comparing mean percent success of crosses for bag types and cross types to test the significance of effects.

Data were also collected on seed traits such as seed number, 1000-seed weight (g), seed width (mm), seed length (mm), germinating seed ratio (%). The analysis was performed using an Anova with Genstat 14 for unbalanced designs.

RESULTS

The two analyses, the one on original percentage of success of crosses and the second on angular transformation did not reveal any discrepancies in results. Therefore, we describe the results of analysis of the original data only. The detailed partitioning in the ANOVA, apart from main factors climatic conditions, cross type and bag type, also included interactions such as: climatic conditions x cross type, climatic conditions x bag type, cross type x bag type. However, none of these interactions were significant. Therefore, we pooled the variance of all interactions with that of error variance. The reduced ANOVA without interactions (Table 1) revealed non-significant effect of climatic conditions on seed set and success of cross. Cross types and bag types, however, had significant effect on seed setting upon pollination.

Partitioning of bag types into orthogonal comparison of **dura**web® vs. both Glassine and Paper-&-Plastic type, and Glassine Vs Paper-&-Plastic type showed the significance of the former comparison. While the Glassine and Paper-&-Plastic types did not differ significantly the PBS International **dura**web® bags were superior to the combined effect of other two types of bags by 12% more success of crosses (Tables 2, 3)

Table 1: Analysis of variance for percent success rate of crosses

Source	df	MS	F	P
Climatic conditions	1	70.73	0.08	0.78
Cross type	1	4593.31	5.33*	0.03
Bag type	1	3180.11	3.69*	0.04
PBS Vs Other types	1	6032.17	7.00**	0.01
Glassine Vs Paper-&-Plastic	1	328.04	0.38	0.54
Error	34	861.70		
Total	38			

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

Mean values for percent success of 45% was the highest with **dura**web® bags which exceeded Glassine by 15% and Paper-&-Plastic type by 7% (Table 2). The Z-test showed a non-significant difference between Glassine and Paper-&-Plastic types. However, the success rate for the PBS International bags was 12% higher than both Glassine and Paper-&-Plastic types together which was highly significant on a Z-test (Table 3).

The success rate of 47% for the intraspecific crosses was 13% higher than the interspecific crosses (Table 2), and this difference was highly significant indicating that seed set will be higher when crosses are made between genotypes of *Miscanthus* that belong to the same species (Table 3).

Table 2. Mean values of percent success of crosses using different type of bags and crosses

Bag type/ Cross type	Number of pairs	Total number crosses attempted in all pairs	Number of successful crosses	Success of crosses (%)	SE (%)
Bag Type					
PBS **dura**web®	18	266	119	44.74	3.05
Glassine	12	180	54	30.00	3.42
Paper-&-Plastic	9	118	45	38.14	4.47
Total	39	564	218	38.65	2.05
Cross type					
Interspecific	27	362	123	33.98	2.49
Intra-specific	12	202	95	47.03	3.51
Total		564	218		

Table 3. Z-test for testing the difference between bag types and types of crosses

Test	Attempted	Successful	Success (%)	Diff (%)	SE diff (%)	Z-value
PBS **dura**web®	266	119	44.74			
Others	298	99	33.22			
PBS duraweb® **Vs Others**				11.52	4.09	2.81**
Glassine	180	54	30.00			
Paper-&-Plastic	118	45	38.14			
Glassine Vs Paper-&-Plastic				8.14	5.63	1.45 NS
Interspecific	362	123	33.98			
Intra-specific	202	95	47.03			
Intra vs inter-specific crosses				13.05	4.31	3.03**

NS= Non -significant; **Significant at 1% probability level.

The three bag types did not differ significantly for most of seed traits. The only significant difference was for 1000-seed weight at 5% level. The mean seed weight was: Glassine bags = 1.55g (n=67), Paper & Plastic = 2.59g (n=48), and PBS = 3.35g (n=125). The SE of difference was 0.739g and least significant difference at 5% = 1.456g. The linear contrast for difference between mean seed weight of Glassine and Paper & Plastic types of bag was not significant. However, the linear contrast PBS bags versus both Glassine and Paper & Plastic bag types was significant. Obviously seeds produced using PBS bag were significantly of higher weight and bolder than the other type of bags. Bolder seeds have higher initial capital and are considered healthier to produce robust seedlings following germination. Such seedlings are known to show better establishment under water stress conditions in the field.

ANALYSIS OF CLIMATIC FACTORS

The general trends for temperature and humidity were the same both inside and outside of the bag (Fig.1). The range of temperature and humidity exhibited by the different bag types were analysed and the results displayed in Figure 2. It can be seen from this chart the range of tempera-ture and humidity found within the PBS International **dura**web® bags compared to the Paper-&-Plastic or glassine bags was much smaller. The tighter control of temperature and humidity demonstrated by the PBS International bags may have had an impact of crossing success and seed set rate.

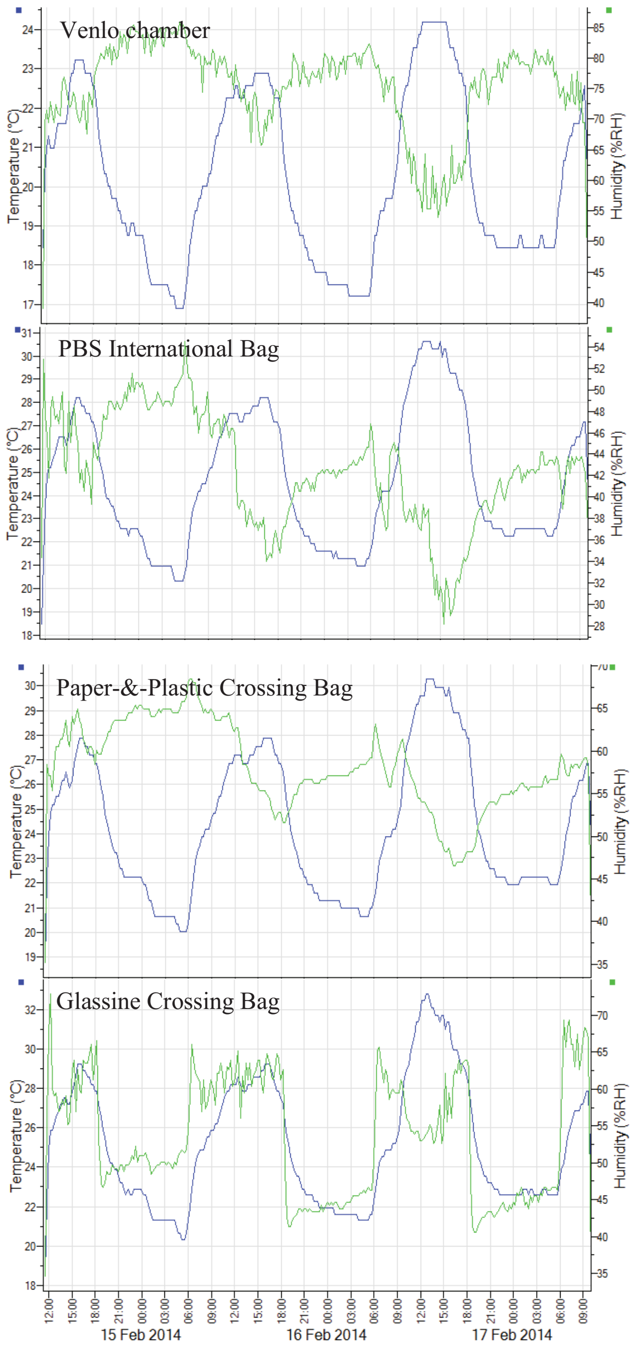

Fig.1. Charts show the humidity and temperature inside and outside a PBS International **dura**web® bag, a Paper-&-Plastic crossing bag and a glassine crossing bag.

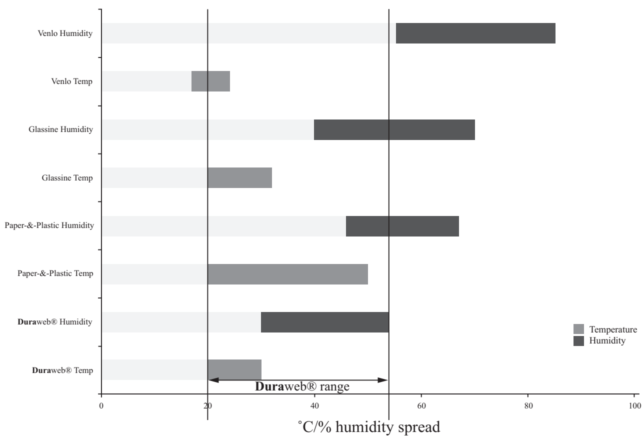

Fig.2. Chart to show the range of temperature and humidity found within the different crossing bags and within the glasshouse.

ECONOMIC ANYALYSIS

Dr Charlotte Hayes, Miscanthus Breeder at the Aberystwyth University, estimated from the institute's data that a cross in *Miscanthus* costs approximately £50. She also considered the relative success rates of crossing programme with different bag types and came up with figures given in Table 4.

Table 4. Comparative costing of a successful cross from different types of bags

Bag Type	No. of crossess attempted	Total cost of crosses	No. of successful crosses	Cost per successful cross	Price compared to Glassine	% relative saving from PBS
Glassine	180	£9,000	54	£167	£0	33%
Paper-&-Plastic	118	£5,900	45	£131	£36 -	15%
duraweb®	266	£13,000	119	£112	£55 -	-

Although the initial outlay of the Glassine bags is much lower it isn't economical when total cost of the cross is considered. The actual cost of the crossing bag is insignificant when factors such as glasshouse space, man hours, storage, consumable, selecting the plant for crossing etc. are considered. Admittedly, the glasshouse costs are high and would be less in an industrial setting but the overall trend would remain the same, and the most economical bags are those of PBS' **dura**web® type which are cheaper by £55 over glassine and £19 over Paper-&-Plastic type for a cross. By using **dura**web® bags there is relative gain of 33% over glassine and 15% over Paper-&-Plastic type.

The relative gain from different bag types for attempting a successful cross was used to simulate the impact of using different bags on production of different number of successful crosses since hundreds to thousands of crosses are attempted breeding programmes. A unit increase in the number of crosses results in £55 savings from **dura**web® bags over Glassine and £19 over Paper-&-Plastic type. The Paper-&-Plastic type had £36 savings over Glassine bags per cross. These increases represent regression coefficients of a linear regression analysis (Fig. 3, Table 5).

Potential impact of use of bag types

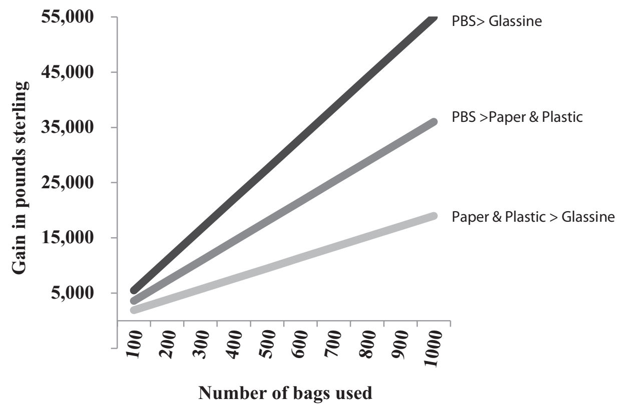

Fig. 3. Relative potential gain in pounds sterling from using PBS' **dura**web® bags

For 1000 crosses, the PBS International bags result in saving of £55,000 over Glassine and £19,000 over the Paper-&-Plastic type of bags. Even when 500 crosses are attempted the saving still remain very significant.

Table 5. Simulated relative gain in pounds sterling for different bag types in comparison to other type of bags with increasing number of crosses

No. of bags	duraweb®> Glassine	dura web® > Paper -&-Plastic	Paper-&-Plastic > Glassine
100	5,500	1,900	3,600
200	11,000	3,800	7,200
300	16,500	5,700	10,800
400	22,000	7,600	14,400
500	27,500	9,500	18,000
600	33,000	11,400	21,600
700	38,500	13,300	25,200
800	44,000	15,200	28,800
900	49,500	17,100	32,400
1000	55,000	19,000	36,000

CONCLUSIONS

The PBS International **dura**web® bags stood up well to the different climatic conditions and were easy to re-use with an autoclave cycle in between. The Paper-&-Plastic bags were unable to withstand an autoclave cycle and so for single use bags were too costly. They were also a bit too short for use with *Miscanthus* species. The glassine bags became brittle under the different climatic conditions and as such were only usable once.

Both of the glassine and the Paper-&-Plastic bags were damaged by slugs and were broken by the continued growth of the *Miscanthus* stems. These problems were not observed in the **dura**web® bags.

The seed set success rates for the different crosses also showed that the **dura**web® bags were much more favourable than for the glassine and Paper-&-Plastic bags also tested throughout the crossing season. Additionally, the seeds produced within PBS bags were bolder and presumably healthier that result in better seedling establishment under stress conditions.

The economic analysis showed that PBS' **dura**web® bags are cheaper by £55 over glassine and £19 over Paper-&-Plastic type per cross with a relative gain of 33% over glassine and 15% over the Paper-&-Plastic type. This economic benefit from using PBS International bags increases as the number of crosses increase and for a moderate number of 500 crosses this translates into £27,500 over Glassine and £9,500 over Paper-&-Plastic type.

Further work is required in order to confirm these results. Self-crosses need to be carried out and repeated intra and interspecies crosses will be required in order to confirm these results during a different crossing season.

(a) cross in a Paper-&-Plastic bag

(b) cross in a PBS' **dura**web® bag

(c) overview of crosses in a Venlo compartment

(d) cross in a glassine bag