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Short Communication

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Testing the Efficiency of Pollination Bag Materials

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With one table

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Abstract

Crossing bags manufactured from three grades of polyester and three types of paper bags were tested for their permeability to grass pollen. Bags were placed on *L. perenne* plants of known *PGI* isozyme genotype. Any pollen passing through the crossing bag and pollinating the plant was detected by assaying its progeny for PGI isozymes. The results obtained allowed differentiation of the various materials for their suitability for pollen proofing.

Key words: *Lolium perenne* — crossing bags — PGI isozymes — pollen proof material

When a cross is made between individual plants of wind pollinated species, the flower heads are enclosed within a pollen proof bag in order to exclude all extraneous pollen. The nature of the bag therefore is of vital importance in the elimination of contamination and the production of F_1 seed; it must be impermeable to any pollen capable of fertilizing the species concerned, and maintain a suitable environment for seed development.

The development of new materials with potential utility in the manufacture of pollination bags requires the establishment of standard tests for determining their efficiency for isolation purposes. Only limited information on the assessment of new pollen proofing materials is available. Griffiths and Pegler (1963) examined a non-woven terylene material and concluded that it provided an effective pollen screen. Further examination of this material by Foster (1968) showed its effect on enhancing seed production compared with traditional glassine bags. The present experiment examines the efficiency of a range of new materials for pollination bag manufacture. The method developed lends itself to more general application for assessing pollen proofing materials.

The technique makes use of isozyme genetic markers in *Lolium* (Hayward and McAdam 1977a) whose ease of assessment makes them ideal for this form of study. The test involves isolating flowering heads of a plant of known isozyme genotype in one of the bags under test and the plant then placed in a pollen proof glasshouse containing plants whose isozyme genotype did not have any alleles in common with that of the bagged plant. The basis of the test was that any seed produced on the heads within the bags could be classified as either selfed or crossed seed, the latter having arisen from pollen which had managed to penetrate the bag.

The plant material used was the cultivar 'Cropper' of *L. perenne* from which were selected desired genotypes at the *PGI/2* (phospho-glucoisomerase, E.C. 5,3,1,9) locus. Heterozygous plants *'ab'*, *'ac'* and *'bc'* were bagged up and placed in pollen proof glasshouses containing groups of plants all having the same homozygous *PGI* genotype namely *'aa'*,

'bb', or *'cc'*. The bagged *'bc'* plants were placed in the *'aa'* house, *'ac'* plants in the *'bb'* house, and *'ab'* plants in the *'cc'* house.

The pollination bags under test were manufactured and provided by Plant Breeding Supplies International, Eastfield Industrial Estate, Scarborough, England, from a new generation of materials specifically selected for plant breeding use. The bags measured 61 × 15 cm and were constructed from six different materials: 1. PBS 10 (a medium grade non-woven polyester); 2. PBS 15 (a heavy grade non-woven polyester); 3. PBS 64 (a lightweight reinforced polyester); 4. PBS L (Latex impregnated paper); 5. PBS 42 (Paper); 6. PBS 42P (Paper and polypropylene). All the bags were translucent and had welded seams except for PBS 64 which had stitched seams. PBS 42P had one side of the bag made of transparent polypropylene. In addition a seventh bag of clear cellophane was used as a control, this being the type customarily used at the Welsh Plant Breeding Station.

Twelve experimental heterozygous plants were used, onto each of which two bags were placed enclosing 5 to 15 flowering heads. Where sufficient heads were available a cellophane bag was also used, in addition one head was emasculated and left out of the bag in order to verify that the plant in the bag and those in the house were cross compatible. It was thus possible to replicate each bag between three and five times randomly across the plants. Each glasshouse received a constant stream of pollen free air.

The bags were left on the plants until the seed had ripened before harvesting. The seed was sown in trays containing a loam based compost. During threshing an estimate of the total number of florets was made in order to calculate percentage seed set. At the two to three tiller stage, leaf material was removed for PGI isozyme determination using a starch gel system (HAYWARD and MCADAM 1977b).

The results of determining the origin of the seeds within each bag type are presented in *Table 1* which shows the total number of seeds and the proportion of selfs and contaminants. Three types of bag, the non-woven polyester of both grades PBS 10 and PBS 15 and the PBS L Latex impregnated paper, produced no contaminants at all, and appear to be impermeable to ryegrass pollen. One contaminant seed was found in the cellophane bags and paper plus polypropylene bags, and three contaminants in the PBS 42 paper only bag. The bags in which most contaminants occurred was PBS 64, made from reinforced polyester, in which 51 were produced. These figures are more relevant presented as the number of contaminant seed per trial, that is per bag, rather than as a percentage of seed set or floret number, both of which can be altered without affecting the number of contaminant seed which are set.

It is assumed that the chances must be very high of fertilization taking place when contaminant pollen manages to penetrate the bag. As can be seen, the incompatibility system in ryegrass is quite efficient and only a low level of selfing occurred, the greatest at 2%. It follows therefore that nearly all florets were unpollinated and thus available to the contaminant pollen grains. The likelihood of contaminant pollen producing seed is therefore high and would not be significantly affected by increasing the number of heads in the bag. (The number of selfed seed is however dependent on the number of heads that are enclosed in each bag.) There might appear to be a correlation between the highest number of contaminants being found in the same bag type as the highest selfing rate, 126 self seed over four plants in bag PBS 64 and 51 contaminant seed. No explanation can be put forward for this result. A one way analysis of variance of the

Table 1. Numbers of contaminant and self seed obtained per bag type

Bag type	No. of bags used (totals)	No. of contaminants	No. of selfs	Total no. of seed	No. contaminants per trial
PBS 10	5	0	83	83	0
PBS 15	4	0	20	20	0
PBS 64	4	51	126	177	12.75
PBS L	3	0	7	7	0
PBS 42	5	3	33	36	0.6
PBS 42P	4	1	70	71	0.33
Cellophane	9	1	32	33	0.11

transformed data of self seed set, showed the variation between bags to be not significantly greater than the variation within bag types. The high rate of selfing observed in the PBS 64 bags is as likely therefore to have arisen by chance as by some causative link with the high incidence of contaminant seed. The flowering heads which had been emasculated produced 100% crossed seed, confirming that the pollen in the house was compatible with the plant in the bags.

The results presented here clearly confirm the efficiency of the non-woven polyester, paper, and paper and polypropylene material for use in crossing bags in the hybridization of grasses and allied species. The use of PBS 64 material for this purpose is however not recommended. *Lolium perenne* has roughly spherical pollen grains with a range in diameter of 26—50 microns (HAYWARD and MANTHRIRATNA 1972), comparable to many closely related species within the Gramineae. As such it must be emphasized that no conclusions can be drawn on the suitability of these bags for use in crossing species with smaller pollen grains.

In order for plant breeders to have confidence in the suitability of pollination bags manufactured from new synthetic materials it is highly desirable that a standard test, such as described here, be adopted. It must be emphasized however, that actual seed set is greatly influenced by environmental conditions within the bag (FOSTER 1968).

Zusammenfassung

Prüfung der Wirkung von Isoliermaterial

Aus neuen Materialien hergestellte Isolierhauben wurden hinsichtlich ihrer Durchlässigkeit gegenüber Gräserpollen geprüft, wobei Isozyme als genetische Markierungen herangezogen wurden. Pflanzen von *Lolium perenne*, deren PGI-Isozym-Genotyp bekannt war, wurden mit je drei verschiedenen Arten von Polyester- und Papierbeuteln eingetütet. Jedes Pollenkorn, das die Isolierhaube durchdrang und zur Befruchtung gelangte, konnte anhand der Nachkommenschaftsanalyse entdeckt werden. Die erhaltenen Ergebnisse ermöglichten es, die unterschiedliche Eignung der verschiedenen Materialien für Isolierungszwecke zu erkennen.

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