Study of reproductive barriers in the production of *P. elliottii* x *P. caribaea* hybrid seed

A SIVLAL[1], S JOHNSON[2] AND A FOSSEY[3]
CSIR Natural Resources and the Environment, PO Box 395, Pretoria, 0001, South Africa
[2]University of KwaZulu Natal, Private Bag 1, Scottville, Pietermaritzburg 3201, South Africa
[3]Central University of Technology, Private Bag 20539, Bloemfontein, 9300, South Africa
Email: asivlal@csir.co.za – www.csir.co.za

INTRODUCTION

Since its introduction, the *P. elliottii* (Pe) x *P. caribaea var. hondurensis* (Pch) hybrid has played a very important role in the plantation forestry industry due to its displayed improved growth, desirable wood properties (Du Plooy, 1984; Malan, 1995) and high level of tolerance to *Fusarium circinatum* (Roux *et al.*, 2007). Currently, there is an unprecedented demand for this hybrid in South Africa.

Research conducted by the CSIR on pine hybrid improvement indicated that the first generation (F_1) Pe x Pch hybrid is difficult to produce as the cross typically results in high abortion rates of cones and low seed yields. The low seed set obtained from the F_1 Pe x Pch cross makes the production of seeds in commercial quantities difficult and costly (Slee, 1970).

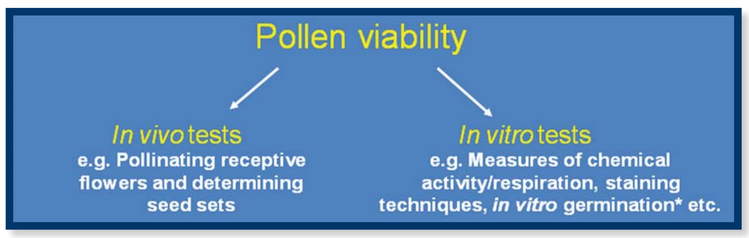
Genetic incompatibilities and poor pollen quality are among the key factors that may be responsible for the high cone abortion rates and low seed yields. The aim of the study was to assess the quality of pollen used in the creation of this hybrid via *in vitro* pollen germination and microscopy studies.

CREATING THE HYBRID



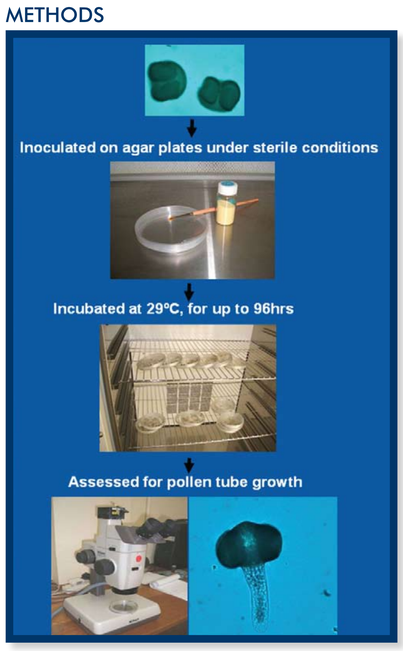
In South Africa, the flowering periods of *P. elliottii* (maternal parent) and *P. caribaea* (paternal parent) are typically three months apart. The pollen of *P. caribaea* is collected and stored until receptivity of *P. elliottii* female cones. The species are hybridised via controlled pollinations: with the first visit the young female cones of *P. elliottii* are bagged (to prevent contamination before receptivity) and then controlled pollinated at the second visit. Several days after pollination the bags are removed and seed cones are collected 20 to 24 months later.

STUDY 1: POLLEN VIABILITY



**In vitro* pollen germination is the most widely used test and was used in this study to determine the quality of pollen

METHODS



In 2007, mature *P. caribaea* catkins from five genotypes were sourced from the Futululu research station in KwaZulu-Natal. *P. elliottii* catkins were obtained from five genotypes located at the CSIR's Mapiep breeding archive in Nelspruit. Upon arrival at the laboratory, the kraft paper bags containing the catkins were spread out in the laboratory to facilitate air-drying. After several days of drying, the pollen was shed from the catkins and sieved. Pollen was dispensed into 50 ml airtight bottles and stored in a desiccator at +/- 4 °C. Prior tests, pollen was dispensed in Petri dishes and suspended over moistened laboratory paper in hermetically sealed buckets for one hour. For each species, a pollen mix was made up by mixing equal quantities of pollen from each of the five genotypes.

Based on other studies, pollen of *P. caribaea* and *P. elliottii* was germinated under aseptic conditions on an agar medium containing a range of sucrose concentrations and on Brewbaker and Kwack Media (BWB) (1963) (**Figure 1**). For each treatment, three replications were conducted. Ten randomly-selected fields of view were captured from each slide. Pollen was scored as germinated when the length of the pollen tube exceeded the width of the pollen grain and was expressed as percent pollen germinated.

RESULTS AND DISCUSSION

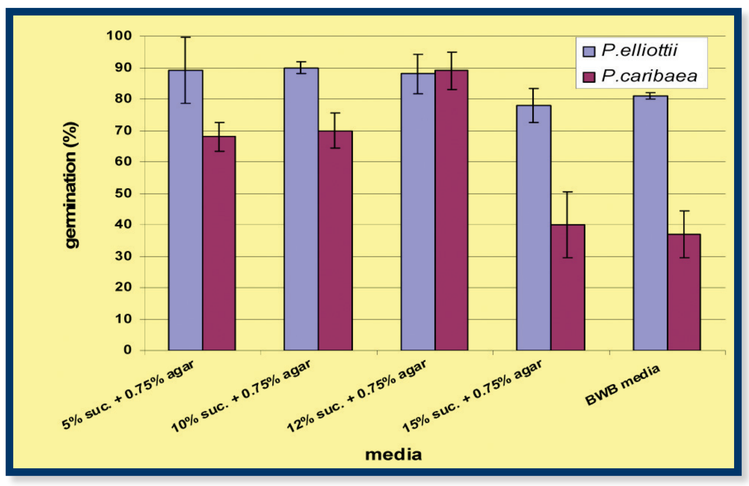


Figure 1: Pollen germination on different media, incubated at 29 °C for 48 hours

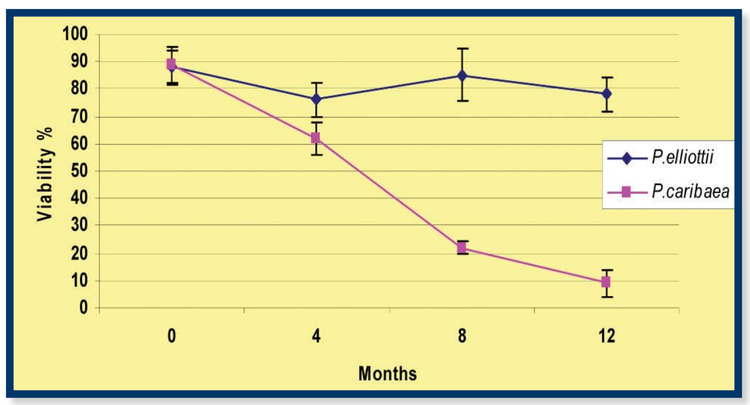


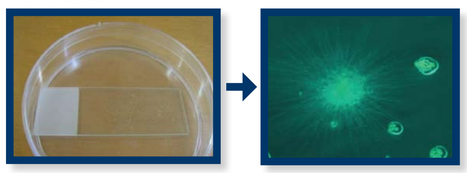
Figure 2: Viability of *P. elliottii* and *P. caribaea* pollen stored at 4 °C

On the different media tested, the highest germination percentage for *P. caribaea* pollen was observed on the 12% sucrose + 0.75% agar medium. For *P. elliottii*, the optimal germination medium was the 10% sucrose + 0.75% agar medium. The viability of fresh pollen from both species was notably high (approximately 90%) (**Figure 1**). The viability of *P. caribaea* pollen decreases steadily within a few months of storage. After a year of storage, the viability of *P. caribaea* pollen is notably low (approximately 10%) (**Figure 2**). Under the same storage conditions, the viability of *P. elliottii* pollen is maintained. These results suggest using *P. caribaea* pollen stored longer than a few months will potentially reduce the seed yields. In the creation of the Pe x Pch hybrid, cone abortions and the production of empty seed are a common occurrence, suggesting that pollen quality is a major factor.

STUDY 2: MICROBIAL CONTAMINATION OF POLLEN

From the *in vitro* germination tests, it was observed that *P. caribaea* germination plates had notably higher levels of fungal growth as compared to *P. elliottii*. In addition, *P. caribaea* pollen appeared clustered and clumpy (picture on the left and in **Figure 3**).

METHODS



Fresh (2008) and stored pollen (2007) of *P. caribaea* and *P. elliottii* (control) was cultured on germination media as illustrated in the previous study and scored for contamination. Pollen of both species was also subjected to Scanning Electron Microscopy (SEM) and sent to the Forestry and Agricultural Biotechnology Institute (FABI) for fungi identification.

RESULTS AND DISCUSSION

Table 1: Differences in *in vitro* contamination of *P. elliottii* and *P. caribaea*

Pollen	no. of plates	no. contaminated
P. elliottii (2007)	25	2
P. elliottii (2008)	25	4
P. caribaea (2007)	25	25
P. caribaea (2008)	25	25
Control (no pollen)	10	0

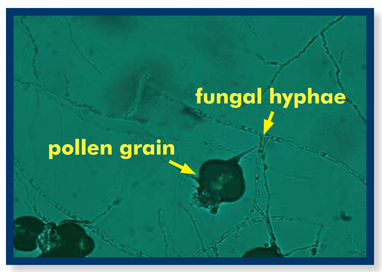


Figure 3: Pollen grains enmeshed by fungal hyphae *in vitro*.

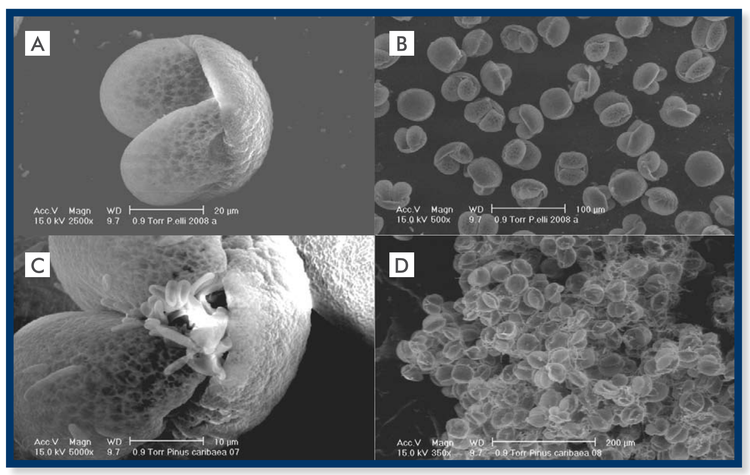


Figure 4: SEM micrographs showing pollen grains of *P. elliottii* (A & B) and *P. caribaea* (C & D) at low and high magnification

Fungi isolated by FABI from *P. caribaea* pollen were predominantly *Alternaria sp.*, *Penicillium sp.* and *Cladosporium sp.* These fungi are responsible for many post-harvest diseases in agricultural crops.

The results suggest that the *P. caribaea* pollen has a high level of fungal contamination. The *in vitro* studies demonstrated that the fungi attack and penetrate pollen grains resulting in many of them bursting (**Figure 4**). The fungi identified by FABI are common post-storage fungi found in seed of many agricultural species. Studies have demonstrated that both *Alternaria* and *Penicillium sp.* produce metabolites which inhibit pollen germination (Hodgkin and Macdonald, 1986; Kimura *et al.* 1991).

CSIR study finds the poor quality of P. caribaea pollen to be a major factor responsible for cone abortions and poor seed yields in the creation of the P. elliottii x P. caribaea hybrid.

The presence of these fungi suggests the moisture content of the *P. caribaea* pollen and storage conditions promote fungal growth. The presence of the fungi is cause for concern as it reduces the viability of the pollen.

FURTHER STUDIES

To determine if fungi have a detrimental effect on pollen germination *in vivo* (microscopy studies), further research is needed to establish a means to inhibit fungal growth by testing different fungicides and optimising pollen harvesting drying procedures and storage conditions.

CONCLUSION

P. caribaea pollen has poor storability under conditions suitable for *P. elliottii* pollen. In addition, *P. caribaea* pollen used in this study had a high level of fungal contamination. The fungal contamination is a major factor contributing to the short storage lifespan of *P. caribaea* pollen. Lastly, the results of the study suggest that the poor quality of *P. caribaea* pollen is a major factor responsible for cone abortions and poor seed yields in the creation of the *P. elliottii* x *P. caribaea* hybrid.

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