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# The effect of temperature and relative humidity on *Acacia mearnsii* polyad viability and pollen tube development

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**Abstract**

*Acacia mearnsii* (black wattle) is a commercially important forestry species in South Africa, grown for its timber and bark. Due to its invasiveness, it is also considered be an alien invader species and for this reason the production of a sterile triploid variety would be highly desirable for South African commercial forestry. Previous research on crosses between diploid and tetraploid parent plants to produce triploid progeny has resulted in poor seed set. One possible barrier preventing seed set could be the effect of temperature and relative humidity, within the isolation bags used during cross-pollination operations. For this reason in 2011 diploid polyads were subjected to various temperature and relative humidity combinations, to simulate conditions recorded within the isolation bags being used in the 2010 flowering season in order to see if the conditions were detrimental to polyad viability. The results showed that when polyads were exposed to extreme temperatures (>30 °C) and low relative humidities (RH's) (10%), polyad viability and pollen tube development, decreased significantly. In contrast the effect of high RH's in combination with low temperatures for long periods appeared to be beneficial to polyad viability and pollen tube growth. The results also indicated that the Australian Centre for International Agricultural Research (ACIAR) agar germination medium was superior to the Brewbaker and Kwack (BK) 30% agar germination medium for determining polyad viability as it resulted in greater number of pollen tubes per polyad, which were healthier in appearance. The Sigma® DAB peroxidase vital stain test overestimated polyad viability and showed no significant differences between the various treatments, highlighting its unreliability as a test. Polyad viability and pollen tube development were compared across three flowering seasons (2009, 2010 and 2011) and similar trends were apparent with some seasonal differences.


*Keywords:* Isolation bags; Polyads; Relative humidity; Temperature

## 1. Introduction

Black wattle (*Acacia mearnsii*) is grown as a commercial forestry species in South Africa with approximately 7.6% of the total area under commercial forestry plantations (Forestry South Africa, 2009). It is grown primarily for the tannins present in the bark and pulp. Native to Australia black wattle is a prolific seed producer in South Africa. Seed can remain dormant in the soil for many years and still retain viability, and for this reason it has been identified as an invasive species in unmanaged stands. The production of seedless or sterile wattle would be beneficial both to the wattle industry and the indigenous vegetation. A sterile variety of black wattle can be produced through the production a triploid, which is created by crossing tetraploids and diploids (Beck et al., 2003).

*A. mearnsii* pollen is grouped together in structures called polyads, with each polyad typically containing 16 pollen grains. The polyads are designed to protect the pollen and ensure that maximum pollen germination is possible when the polyad attaches to the stigma (Kenrick and Knox, 1982). One of the reproductive barriers limiting cross-pollinated seed production could be the viability of the pollen used in cross pollinations. Stiehl-Alves and

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Martins-Corder (2007) recorded that poor development of viable male gametes was a limiting factor contributing to the low seed set in *A. mearnsii*. In a study conducted by Beck-Pay (2011a) in vitro agar media germination tests [Australian Centre for International Agricultural Research (ACIAR) and modified Brewbaker and Kwack (1963) (BK) media] together with vital stain tests (Sigma® DAB peroxidase and *p*-phenylendiamine) were used to test *A. mearnsii* polyad germination and viability. These were then compared to *in vivo* polyad germination on the stigma (Beck-Pay, 2012a). Results showed that the vital stain tests gave significantly ($p < 0.05$) higher polyad viability (59.53% and 60.67%) than the agar germination tests (11.92% and 24.50%) and were more in agreement with the results from the polyad germination rate on the stigma (94.59%) (Beck-Pay, 2012a,b). The conclusion from these studies was that polyad viability did not appear to be a restriction in seed set, but rather low pollination rates (polyads adhering to the stigma) and that in the cross to produce a triploid (2n × 4n or 4n × 2n), the diploid polyads were significantly more vigorous and suitable in fertilising the tetraploid ovaries as opposed to the reverse. A further barrier however, that could be affecting diploid polyad viability and contributing to low seed set, could be a suboptimal microclimate within the isolation bags used during cross-pollination operations.

The combined effect of temperature and relative humidity (RH) on pollen viability cannot be disregarded in reproductive biology studies where optimization of seed production is critical. Different plant species react differently and have variable tolerances to high and low RH (Bassani et al., 1994). Baltazar and Schoper (2002) noted that as temperature increased, so the RH decreased resulting in a decrease in pollen viability. Shivanna et al. (1991) however, recorded that under conditions of high RH's, the hydration of the pollen activates some physiological process making the pollen susceptible to heat stress. A study on *Myrtus communis* pollen by Aronne (1999) showed that pollen viability remained high under low RH's and high temperatures but decreased rapidly with increasing RH. These studies not only show the variability between species to temperature and RH but also indicate that RH could be the critical factor in determining pollen viability. The positive effect of high RH on pollen germination and pollen tube growth has also been recorded with avocado (Marianthi et al., 1997), papaya (Cohen et al., 1989), walnut (Luza and Polito, 1987) and lily (Simons et al., 1972). Studies conducted by Baltazar and

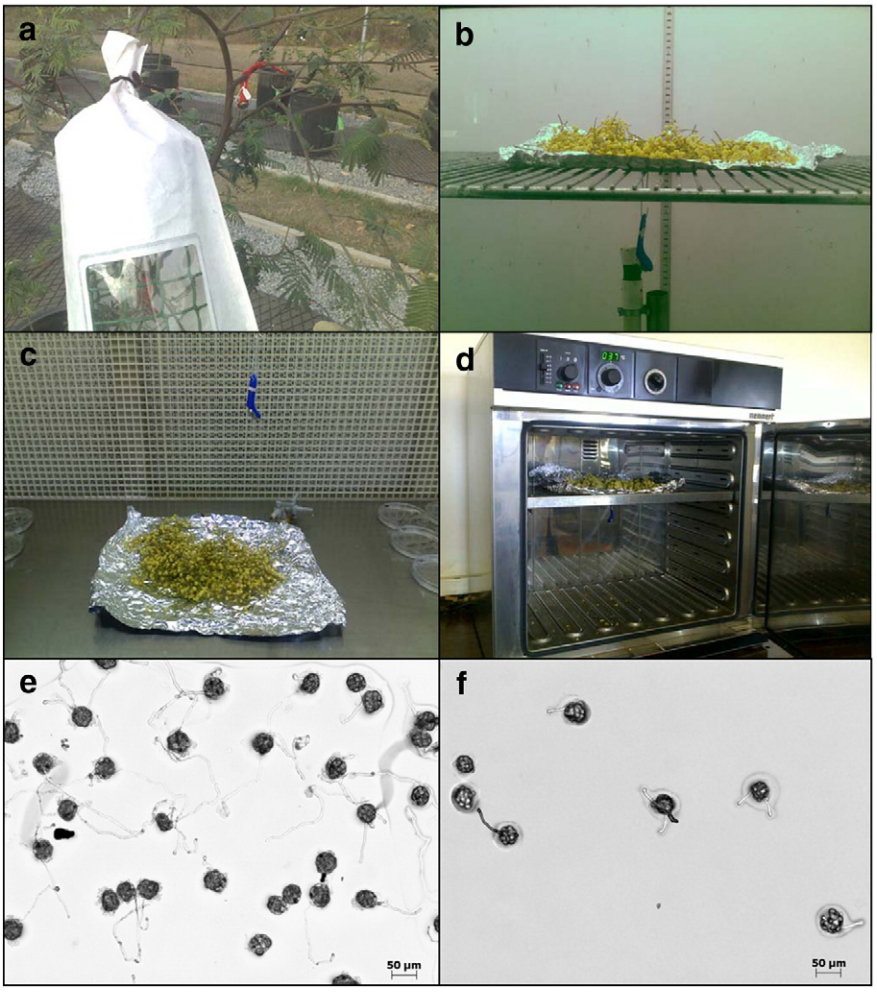


Fig. 1. Determination of *A. mearnsii* polyad viability at different temperatures and relative humidities. (a) iButtons were used to monitor the temperature and relative humidity within isolation bags. Inflorescences were placed either in a (b) conviron, (c) on a laminar flow bench, or (d) in an oven at a set temperature and relative humidity. Agar germination of polyads on (e) ACIAR and (f) BK 30% sucrose medium.