



Self-pollination and inbreeding depression in *Acacia dealbata*: Can selfing promote invasion in trees?



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ABSTRACT

The ability to self-fertilise may promote invasiveness in plants by assuring reproduction when mate and pollinator availabilities are inadequate, provided that the benefit of increased fecundity via selfing is not outweighed by inbreeding depression. However, knowledge of breeding systems and inbreeding depression has been lacking for most introduced plants. In this study of the invasive Australian tree *Acacia dealbata* in its introduced range in South Africa, controlled pollination experiments indicated that the study population was at least partially self-compatible and had a high capacity for autonomous self-pollination. However, we found substantial inbreeding depression, with seeds per fruit, progeny survival and progeny growth being lower after self- than after cross-pollination. Progeny arising from self-pollination also had a higher frequency of certain traits – yellow colouration of leaves and pink or white colouration of stems – which were associated with lower rates of survival. High inbreeding depression in *A. dealbata* must detract from the reproductive assurance benefit of self-fertilisation, casting doubt on the hypothesis that self-fertilisation contributes to invasiveness in this species. As high inbreeding depression has also been reported in other self-compatible trees, future studies should elucidate whether self-fertilisation contributes to invasiveness of trees by assessing both reproductive assurance benefits and inbreeding depression costs over the lifetime of progeny.

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1. Introduction

To become invasive, introduced plants must be able to reproduce in their novel range. It has been predicted that the ability to self-fertilise should promote invasiveness in introduced plants by assuring reproduction when mates and pollinators are in short supply (reproductive assurance) (Baker, 1965a, 1974; Barrett, 2011). In line with this, several studies have found positive associations between invasiveness and selfing ability (Van Kleunen and Johnson, 2007; Van Kleunen et al., 2008; Burns et al., 2011; Hao et al., 2011; Pyšek et al., 2011). However, inbreeding depression – reduced performance of inbred relative to outbred progeny – is very common in plants and detracts from the reproductive assurance benefits of self-fertilisation (Husband and Schemske, 1996). Self-fertilisation can actually be detrimental to reproductive success when inbreeding depression is present and pollinator visitation and mate availability make outcrossing possible. In such cases, inferior selfed progeny may be produced at the expense of fitter, outcrossed progeny (seed discounting, Lloyd, 1992). When considering whether selfing may promote invasion in plant species, it is therefore important to take inbreeding depression into account. Although many invasive species are now known to self-fertilise (Van

Kleunen and Johnson, 2007; Van Kleunen et al., 2008; Burns et al., 2011; Hao et al., 2011; Pyšek et al., 2011), there are very few investigations of inbreeding depression for introduced species (although see Ward et al., 2012; Mullarkey et al., 2013).

Studies of plants in the native range have shown that selfing ability and inbreeding depression vary between life forms. While herbaceous plants show a wide range of breeding systems and levels of inbreeding depression, woody plants in general and trees in particular, are predominantly self-incompatible with high levels of inbreeding depression (Duminil et al., 2009). This suggests that even when they do produce seeds by self-fertilisation (Barrett et al., 1996; Husband and Schemske, 1996; Duminil et al., 2009), woody plants may not realise benefits from reproductive assurance (Lande et al., 1994; Morgan, 2001). Trees generally have higher outcrossing rates than herbaceous plants (measured from seeds or seedlings), probably because high levels of inbreeding depression select against traits that promote selfing (Barrett et al., 1996; Scofield and Schultz, 2006; Duminil et al., 2009). In fact, very few woody plants are known to be fully self-compatible or to have predominantly selfing mating systems (Barrett et al., 1996 but see Hardner and Potts, 1995, 1997; Ishida, 2006, 2008; Robertson et al., 2011).

High inbreeding depression in trees may be a result of their size – as a greater number of mitotic cell divisions should result in more mutations (Scofield and Schultz, 2006) – or their longevity – as the cumulative effects of inbreeding depression increase throughout life (Morgan, 2001). In trees and shrubs, large floral displays often

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encourage pollinators to visit multiple flowers on the same plant consecutively, resulting in high rates of geitonogamous self-pollination (De Jong et al., 1993), which will result in seed discounting in self-compatible plants. Therefore, for woody plants, it is particularly important to consider inbreeding depression when assessing the potential of selfing to provide a demographic advantage through reproductive assurance.

We aimed to assess whether self-fertilisation potentially contributes to invasiveness in the Australian tree *Acacia dealbata* in its introduced range in South Africa. Specifically, we assessed self-compatibility by comparing fruit set after cross, self, and natural pollinations and evaluated inbreeding depression from subsequent seed development, seedling growth and survival of progeny produced by these pollination treatments.

2. Materials and methods

2.1. Study species and study population

A. dealbata (silver wattle) invades grassland and forest habitat in the moist eastern summer rainfall regions of South Africa (Henderson, 2007), growing either as a tree or shrub, depending on environmental conditions. It originates in temperate south-eastern Australia, as a forest pioneer (Sherry, 1971) and an element of grassy woodland communities (Broadhurst and Young, 2006). Extensive plantations of *A. dealbata* were established in KwaZulu-Natal, South Africa in the nineteenth century, and it remains an important source of fuelwood, although it is no longer grown commercially (Poynton, 2009).

In KwaZulu-Natal, *A. dealbata* mass-flowers in early spring (July to September). It bears racemes of compact yellow globose flower-heads (inflorescences) containing 22–42 minute protogynous flowers (Broadhurst and Young, 2006) (Fig. 1). These inflorescences are highly integrated units that act as blooms and produce only one or a few fruits each (Kenrick and Knox, 1989a; Gibson et al., 2011). In Australia *A. dealbata* is mainly diploid, with occasional tetraploids and triploids reported (Blakesley et al., 2002). Allozyme analysis indicates that this species is highly outcrossing in its native range ($t_m = 0.89$ – 1.00 , $n = 6$ populations), where it is probably pollinated by introduced honeybees and native bees (Bernhardt,

1989). Prior to this work there was no information on the reproductive biology of *A. dealbata* in its introduced range in South Africa, but a sister study indicated that honeybees are its main pollinators in the study region (Rodger, 2012).

The population studied was a stand of mature trees, about 20 by 200 m along a watercourse on the farm Dalcrue, near Nottingham Road in the KwaZulu-Natal Midlands ($29^{\circ} 22.9' S$, $29^{\circ} 54.5' E$, 1500 m ASL). Although information on age, number of founders and other aspects of history of the stand was not available, expression of strong inbreeding depression indicates that the population is not genetically homogeneous (see Discussion).

2.2. Controlled pollinations

Controlled pollination experiments, conducted between the 6th and 18th of August 2004, were used to assess the ability of *A. dealbata* to self-fertilise in its introduced range. Four treatments were applied to 16 trees: hand cross-pollination, hand self-pollination, autonomous self-pollination and natural pollination. Nylon mesh bags were used to exclude floral visitors from all except naturally pollinated branches. After bagging, autonomous self-pollination treatments were not further manipulated. All inflorescences which had already opened were removed from treated branches at the start of the experiment. Hand cross- and hand self-pollinations were applied to one branch per tree; autonomous self-pollination treatments to four branches per tree and three branches per tree were marked for natural pollination. More branches were used for the autonomous self and naturally pollinated treatments to ensure that sufficient fruit were obtained for comparison of seeds per fruit between treatments.

Wire spirals were attached to branches prior to bagging to minimise contact between flowers and the bag, except that for autonomous self-pollination, two bags per tree had wire spirals and two bags did not. This was to assess whether contact between flowers and the bag would promote self-pollination. A paired samples t-test showed that fruit set (arcsine-square root transformed) did not differ between autonomous self-pollination branches with and without wire ($t = 1.272$, $df = 10$, $p = 0.232$), so data were pooled. Data were also pooled for all naturally pollinated branches on each tree. A median of 81 inflorescences was used per branch over all treatments (minimum = 11, maximum = 337).

Pollen for cross-pollination was obtained from a tree at least 20 m away, which ensured that pollen donors and recipients were separated by a few intervening plants. Due to intensive honeybee foraging, branches were bagged to ensure availability of pollen. Pollen was applied by rotating donor inflorescences against recipients and treated inflorescences were marked on the stalk with a Sharpie Pen (Sanford™). The proximity of stigmas to anthers makes it likely that some self-pollination occurred in all treatments. However, emasculation to prevent self-pollination is not practical in these plants due to the compact nature of inflorescences and small size of flowers. All inflorescences which opened on self and cross-pollinated branches during the experiment were pollinated on their first day and again a day later.

Fruit set was scored just prior to maturity in November 2004, as proportion of inflorescences that set at least one fruit. Fruit-set is frequently scored in this way in *Acacia* due to the highly integrated nature of inflorescences (Kenrick and Knox, 1989b; Gibson et al., 2011; see Fig. 1). Early inbreeding depression was assessed from seeds per fruit. Treated branches were bagged prior to maturity to prevent loss of seeds and fruits due to abscission and dehiscence. Number of full seeds per fruit was scored when fruits were collected at maturity in December that year. Seeds were regarded as full if they were swollen and if they did not break when held between the fingernails and squeezed gently. As some fruits had dehisced in the bags prior to scoring, we obtained mean number of seeds per fruit as number of seeds/number of fruits, pooling all branches for each treatment on each tree.



Fig. 1. Flowering branch of *Acacia dealbata*. Flower heads are ± 5 mm in diameter and consist of 22–42 individual flowers. Styles can be seen protruding beyond the anthers. Photo A. Shuttleworth.

2.3. Progeny trials

Seeds from the breeding system experiment on *A. dealbata* were sown on the 14th of April, 2007 at the University of KwaZulu-Natal, Pietermaritzburg. Seeds were nicked with a razor blade to remove a small piece of the seed coat and placed randomly in separate cells in eleven germination-trays (128 cells per tray) filled with a mixture of composted pine bark and coco fibre (Top Crop™ wattle mixture). Seedlings were maintained in a shade-house to an age of three months. Germination, survival and growth were assessed for progeny from self-, cross- and natural pollination in order to assess inbreeding depression and its impact on performance of progeny produced by natural pollination. Germination was scored at one month (only three dead seedlings were recorded and no germination was observed subsequently) and the presence of live seedlings (cumulative survival) three months after sowing. Seedling height was measured to within 1 mm after one month and again after 3 months from sowing. Growth was calculated as (height at three months – height at one month).

Certain unusual characters were apparent in the seedlings and we scored their presence at the same time as germination, in order to assess whether they were associated with reduced performance. Predominantly yellow and slightly yellow variants were observed for leaf colour, as opposed to the normal green. Red colouration was observed, either visible on the leaf margin and the dorsal surface of the leaf edging the margin, or only on the margin itself, although most leaves lacked any red colouration. Stem colour variants were either predominantly white or pink, as opposed to the normal green.

2.4. Statistical analyses

Breeding system was assessed by comparing differences between treatments in proportion fruit set in ANOVA in SPSS 15 (SPSS, 2008). Proportion fruit set for each treatment on each tree was arcsine-square root transformed and tree was included as a factor in the analysis. Tukey posthoc tests were used to compare pairs of treatment means in all analyses.

Early inbreeding depression was assessed from seeds per fruit. *Acacia* is particularly suited to this because stigmas usually receive a single polyad (Kenrick, 2003; Moncur et al., 1991), minimising noise associated with variation in quantity of pollen received, so seeds per fruit depends almost entirely on pollen quality (outcross versus self). Data from autonomous and hand self-pollinations were pooled for inbreeding depression analysis as sample sizes were low and because these treatments are genetically equivalent. Although these treatments might differ in seeds per fruit if there was a higher level of fruit-set in one treatment and a trade-off between proportion fruit-set and seeds per fruit, no significant difference was found in fruit-set (Fig. 2) or seeds per fruit (independent samples t-test: $t = 0.47$, $df = 15$, $p = 0.643$). Crossed, naturally pollinated and selfed treatments were compared using REML analysis of variance in Genstat as sample sizes were unbalanced. Rank-transformed data was used as no other transformation provided acceptable homogeneity of variances. Although rank-transformation can result in type I error rates above the nominal level for interaction terms (Seaman et al., 1994), we did not include an interaction term in this model. Tree was included as a random effect and differences between pairs of treatments were assessed with Fisher's unprotected least significant difference test. Although corrections for multiple comparisons are sometimes applied for pairwise comparisons, we have not done so in this case because each comparison tests a different hypothesis, so the multiple comparisons do not inflate type 1 error. Significance of the fixed effect (treatment) was evaluated from the Wald F-statistics and significance of the random effect (tree) was evaluated by comparing change in deviance in the model when the term was dropped to a chi-squared distribution with one degree of freedom (Payne et al., 2011).

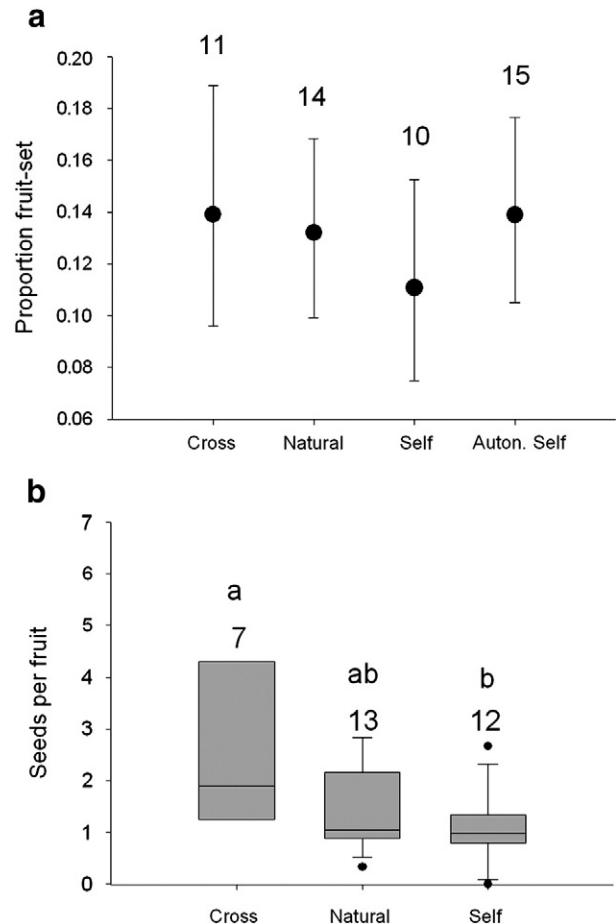


Fig. 2. Proportion fruit set (infructescences per inflorescence) (a) and full seeds per fruit (b) in a controlled pollination experiment on *Acacia dealbata*. Numbers above bars represent sample sizes (number of plants). Back-transformed means \pm standard errors from arcsine-square root transformed data shown in (a). Treatments are significantly different where they do not share a letter in (b). In (b) whiskers indicate 10th and 90th percentiles where sample size is large enough to calculate them. In (a) 'Cross' and 'Self' denote hand pollination treatments and 'Auton. self' represents autonomous self-pollination (bagged to exclude pollinators and not hand pollinated). In (b) hand self- and autonomous self-pollination treatments are pooled under 'Self'.

An index of self-incompatibility ($ISI = 1 - \text{fruit set for self-pollination} / \text{fruit set for cross-pollination}$, Raduski et al., 2012 after Lloyd, 1965) was calculated using untransformed fruit set data (proportion of inflorescences setting fruit). Means and confidence intervals were generated by bootstrapping with replacement, 10000 times using PopTools Add in for Excel (Hood, 2009). Fruit-set values were available from 11 trees for cross-pollination and nine for self-pollination. In each round, resampling with replacement, 11 values were drawn for cross-pollination and nine values for self-pollination. The means for cross- and self-pollinations were used to calculate ISI in each round, and the bootstrapped mean and confidence intervals were generated from the 10000 rounds. We take ISI values of less than or equal to 0.2 to indicate self-compatibility, 0.2–0.8 partial self-incompatibility and greater than or equal to 0.8 self-incompatibility (Raduski et al., 2012). An autofertility index (AFI) was calculated from fruit set as autonomous self-pollination/hand self-pollination (Lloyd and Schoen, 1992; Eckert et al., 2006) with means and 95% confidence limits calculated by bootstrapping as for ISI. Where both hand cross- and self- or hand cross- and autonomous self-pollination treatments were available for the same tree, ISI and AFI values were calculated for each individual to examine potential variation in ability to self-fertilise.

Early inbreeding depression was calculated from medians of tree values for seeds per fruit for selfing (pooling hand self- and autonomous

self-pollination) and outcrossing using the formula $\delta = 1 - \text{performance of selfed progeny} / \text{performance of outcrossed progeny}$ (Husband and Schemske, 1996).

Germination, survival from one to three months and cumulative survival from sowing to three months (i.e. inclusive of germination) were evaluated in generalised linear mixed models in R 2.12.0 (R Core Team, 2010) with the lmer function in the lme4 package, using Laplace approximation (Bates and Maechler, 2010). Germination-tray and tree were included as random effects and pollination treatment as a fixed effect. Significance of random effects was tested by dropping them one at a time from the full model, and testing change in log-likelihood against the chi-squared distribution with one degree of freedom. z-Values from the full model were used to evaluate whether performance of progeny from self- and natural pollinations was lower than that for cross-pollination.

Inbreeding depression was assessed for height at one month using REML analysis of variance in Genstat 12 (Genstat, 2009). Pollination treatment was included as a fixed effect and germination-tray and tree were included as random effects in analyses for both height measurements. For height at three months, analysis was conducted first exactly as for height at one month, but an additional analysis was conducted, in which height at one month was also included as a fixed effect. Growth between one and three months was analysed with the same effects as height at one month. Significance of fixed effects was evaluated using Wald F-statistics. Treatments were compared using Fisher's unprotected least significant difference (ULSD) tests to compare pairs of treatments. For random effects – tree and germination-tray – significance was evaluated by dropping terms one at a time from the full model, and comparing the change in deviance to a chi-squared distribution with one degree of freedom (Payne et al., 2011). Estimates of Inbreeding depression (δ) were calculated for germination, survival and height as for seeds per fruit. Back-transformed predicted values from generalised linear mixed models (employing logit transformation) were used to calculate δ for germination and cumulative survival. Predicted values from REML analysis of variance were used to calculate δ for height. Cumulative inbreeding depression was calculated as $1 - (1 - \delta_{\text{seeds per fruit}}) \times (1 - \delta_{\text{cumulative survival}}) \times (1 - \delta_{\text{height 1 month}}) \times (1 - \delta_{\text{height 3 months}})$. Values used to calculate $\delta_{\text{height 3 months}}$ were obtained while controlling for height at one month.

We tested whether the frequencies of seedling traits (leaf and stem colouration) differed among pollination treatments, again using generalised linear mixed models with Laplace approximation in R, as described above for germination and survival, as data were binomial. The response variable in each case was the presence or absence of the relevant character: e.g. all seedlings with predominantly yellow leaves coded 1 and all others coded zero. Pollination treatment was included as a fixed effect and germination-tray and tree as random effects. Further, we tested whether these unusual characters were associated with reduced survival from germination to three months in a generalised linear mixed model with Laplace approximation. Leaf yellowness, leaf redness, and stem colour were included as fixed effects, each with three levels, pollination treatment was also included as a fixed effect and tree and germination-tray were included as random effects.

Assumptions of analyses were assessed from qq plots and plots of residuals against fitted values for ANOVA and REML analysis of variance. For generalised linear mixed models with Bernoulli (0 or 1) data such as those presented here, there are no clear guidelines for interpretation of residuals and overdispersion cannot occur (Zuur et al., 2009, p 253). Although previous studies of *A. dealbata* estimated minimum outcrossing rates using seedling characters which segregated in 3:1 and 15:1 ratios (Philp and Sherry, 1946; Moffet, 1956), we were unable to do so as there was variation for each trait examined within the majority of progeny families (offspring from the same maternal plant), including within progeny from cross-pollination. The necessary assumption that all individuals showing rare characters came from selfing therefore seems rather unrealistic.

3. Results

3.1. Controlled pollinations

In *A. dealbata* fruit set (proportion of inflorescences setting fruit) did not differ significantly among pollination treatments ($F_{3, 32} = 1.04$, $p = 0.387$; Fig. 2a), although there was significant variation among trees ($F_{14, 32} = 6.90$, $p < 0.001$). The index of self-incompatibility (ISI) estimated from proportion fruit set was 0.22, classifying *A. dealbata* as partially self-compatible (in the range 0.2–0.8), although the confidence limits – lower confidence limit (LCL) = -0.43 , upper confidence limit (UCL) = 0.63 – include the range for self-compatibility (0–2). The autofertility index (AFI) was 1.44 (LCL = 0.80, UCL = 2.56) indicating that *A. dealbata* is highly autonomously self-pollinating (a value of 1 indicates identical fecundity under hand-self and autonomous self-pollinations and is the theoretical upper limit for AFI; although the mean value here is greater than, 1 the confidence limits include 1, so this is not biologically or statistically significant). ISI values could be calculated for eight individual trees with median = 0.07 and range -0.5 – 1 ; AFI values could be calculated for eight trees with median = 1.15 and range 0.54–2.53.

Pollination treatment had a significant effect on seeds per fruit ($F_{2, 28.1} = 3.39$, $p = 0.048$; Fig. 2b), with median values of 1.89 seeds for cross-pollination, 0.98 seeds for self-pollination and 1.04 seeds for natural pollination (Fig. 2b). Fisher's unprotected least significant difference test indicated that cross-pollinated fruits had significantly more seeds than self-pollinated ($p = 0.015$) but natural versus self ($p = 0.208$) and natural versus cross ($p = 0.137$) comparisons were non-significant (Fig. 2b). Inbreeding depression for seeds per fruit was estimated as $\delta = 0.481$ from median values.

3.2. Progeny trials

Inbreeding depression was apparent in progeny trials, but only became significant at three months from sowing (Tables S1–S6; Fig. 3). Germination was between 80 and 90% for all treatments and was not significantly greater for seeds from cross-pollination than self-pollination ($p = 0.123$) or natural pollination ($p = 0.100$) (Table S1; Fig. 3a). Height of seedlings at one month did not differ significantly between treatments (Wald- $F_{2, 1033.7} = 1.75$, $p = 0.174$), although the rank order of the treatment means – cross > natural > self – was consistent with inbreeding depression and mixed mating under natural pollination (Table S2; Fig. 3b). Selfed seedlings had significantly lower survival from one to three months than crossed seedlings ($p = 0.020$) although the difference between natural and cross was not significant ($p = 0.278$) (Table S3, Fig. 3c). Growth from one to three months did not differ significantly between treatments (Wald- $F_{2, 702} = 2.44$, $p = 0.088$), with rank order of treatments being natural > cross > self (Table S4, Fig. 3d). Cumulative survival to three months was significantly lower for progeny arising from self-pollination compared to those arising from cross-pollination ($p = 0.004$), with a trend for higher survival for progeny from cross-pollination than natural pollination ($p = 0.089$; Table S4; Fig. 3e). Inbreeding depression (δ) for cumulative survival was 0.28. Treatments differed significantly in seedling height at three months (Wald- $F_{2, 830} = 4.12$, $p = 0.017$; δ for height at three months = 0.06) with the rank order of treatments being cross > natural > self (Table S5; Fig. 3f). However, when height at one month was included as a covariate, then height at three months did not differ significantly between treatments (Wald- $F_{2, 667} = 2.78$, $p = 0.063$). Cumulative inbreeding depression was estimated as 0.65 from pollination to three months and 0.32 from sowing to three months.

Seedlings arising from self- and natural pollinations had a higher frequency of predominantly yellow leaves than those arising from cross-pollination, although only self-pollination was significant (Table S7: $p = 0.046$ and $p = 0.070$ respectively). The frequency of slightly

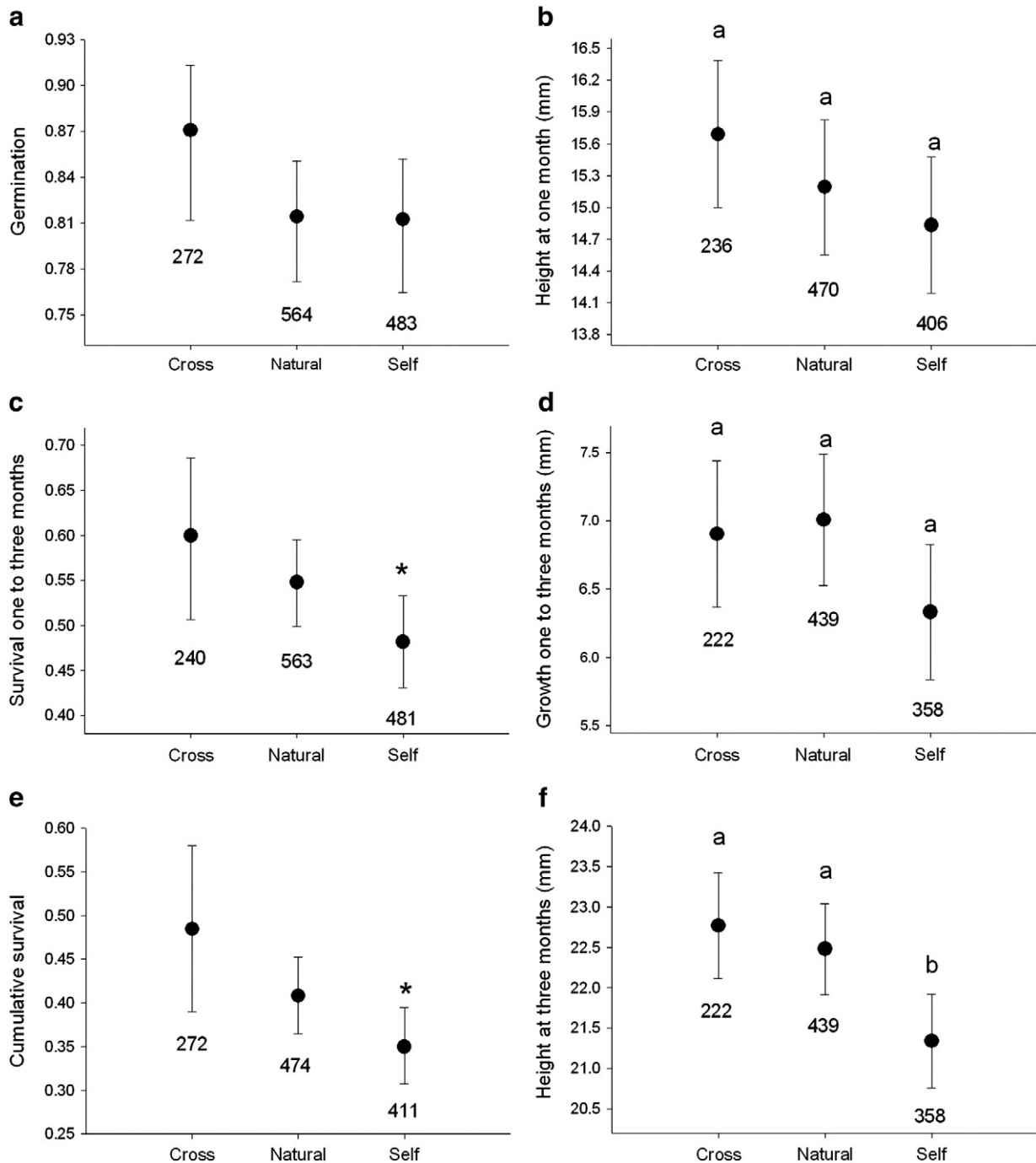


Fig. 3. Performance of progeny from cross-, self- and natural pollinations. Proportion germination at one month (a); height of seedlings at one month (b); survival from one to three months (c), growth from one to three months (d), cumulative germination and survival (i.e. proportion of seeds with live seedlings) at three months (e); and height at three months (f). Bars represent predicted means and standard errors from statistical models; numbers below bars represent number of progeny assessed. Progeny represent seven trees for cross-pollination, ten for natural pollination and nine for self-pollination in germination and survival analyses (a, c, e) and seven, nine and nine trees respectively for growth and height. For germination and survival (a, c, e) * indicates a treatment that is significantly different from cross-pollination ($p < 0.05$); for growth and height (b, d, f) treatments not sharing a letter above the bar are significantly different, as assessed by Fisher's unprotected least significant difference tests.

yellow leaves did not differ between pollination treatments (Table S8: self versus cross, $p = 0.295$; natural versus cross, $p = 0.93$). Pollination treatment also did not affect the frequency of seedlings with red colouration on both the dorsal surface and margins of leaflets (Table S9: self versus cross, $p = 0.179$; natural versus cross, $p = 0.293$) or with red colouration only on margins (Table S10: versus cross, $p = 0.253$; natural versus cross, $p = 0.916$). There was a higher frequency of white stems among seedlings from self- and natural compared to cross-pollination but this was only significant for self-pollination (Table S11:

$p = 0.003$ and $p = 0.161$ respectively). There was a significantly higher frequency of pink stems among seedlings from self- and natural compared to cross-pollination (Table S12: $p = 0.018$ and $p = 0.049$ respectively). All these traits varied significantly among maternal trees ($p < 0.05$; Tables S7–S12).

Leaf yellowness and stem colour were significantly associated with lower rates of survival between germination and three months, but leaf redness was not (Table S13). Seedlings with predominantly yellow leaves had dramatically and significantly lower survival between

germination and three months than green-leaved seedlings (5.5% as opposed to 56.5%, $p < 0.001$), but slightly yellow-leaved seedlings did not differ from green-leaved seedlings in survival (47.2% versus 54.2%, $p = 0.489$). Survival of seedlings with white stems (15.9%) and pink stems (46.6%) was significantly lower than for green-stemmed seedlings (59.5%); ($p < 0.001$ and $p = 0.002$ respectively).

4. Discussion

The *A. dealbata* population examined was at least partially self-compatible (ISI = 0.22) and had a high capacity for selfing autonomously (AFI = 1.44). There were no significant differences in fruit set between pollination treatments (Fig. 2a) and the ISI confidence limits included 0, the value for complete self-compatibility. This is unusual, but not unprecedented in *Acacia*. Three out of ten other species in *Acacia* s.s. (previously subgenus *Phyllodineae*) for which controlled pollination experiments have been conducted can be considered at least partially self-compatible: *Acacia ulicifolia* (ISI = 0.04), *Acacia paradoxa* (ISI = 0.14) and *Acacia saligna* (ISI = 0.26) (Kenrick and Knox, 1989b; Gibson et al., 2011, ISIs recalculated following Raduski et al., 2012). The rest were strongly self-incompatible (ISI > 0.8: Moffett and Nixon, 1974; Bernhardt et al., 1984; Kenrick and Knox, 1989b; Morgan et al., 2002, summarised in Gibson et al., 2011).

Substantial inbreeding depression was detected in *A. dealbata*, over seed development and progeny growth and survival to three months, with a cumulative value of $\delta = 0.65$. Seeds per fruit (Fig. 2b) and germination of seeds (Fig. 3a) from natural pollination were similar to those from self-pollination, suggesting a mixed mating system with relatively high rate of selfing. Over the course of the trial, performance of progeny arising from natural pollination became more similar to those from cross-pollination (Fig. 3a–d), presumably due to higher mortality of selfed than outcrossed progeny among them. Height at three months differed significantly between treatments (Fig. 3f) only when height at one month was not included as a covariate and growth from one to three months did not differ significantly between treatments (Fig. 2d). These results indicate that inbreeding depression accumulates from one to three months. The presence of inbreeding depression also indicates that there was reasonable genetic diversity in the study population. If genetic diversity was low, then shared deleterious alleles would make performance of progeny from cross-pollination similar to that of selfed progeny (Angeloni et al., 2011).

Although controlled pollinations and progeny trials suggest a high rate of selfing in the population investigated in this study, Australian populations of *A. dealbata*, have been found to be highly outcrossing, with selfing rates measured from 0 to 0.11 in isozyme analysis of seedling progeny arrays from six populations (Broadhurst et al., 2008). In plants that have very large floral displays, such as *A. dealbata*, insects move mostly between flowers on the same plant, bringing about high rates of geitonogamous self-pollination (De Jong et al., 1993). Where self-compatibility is present, this will cause high rates of self-fertilisation. Therefore, the low selfing rates of Australian populations of *A. dealbata* strongly suggest that they are self-incompatible. Unfortunately, direct comparisons are not possible as controlled pollinations have not been conducted in Australia and isozyme analysis has not been conducted in South Africa. Few comparisons of self-compatibility between native and introduced ranges are available in general. To the best of our knowledge, the only species shown to have higher self-compatibility in the introduced than the native range are short lived shrub *Gomphocarpus physocarpus* (Ward et al., 2012; Coombs et al., 2009) and the annual *Echium vulgare* (Petanidou et al., 2012).

One explanation for increased self-compatibility in the introduced range is preferential introduction of self-compatible genotypes. This is plausible for *A. dealbata*, as *Acacia* species frequently display variation in self-compatibility (Kenrick and Knox, 1989b) and the range of values of ISI (0–1.5) and AFI (0.17–2.58) for individual trees in our study population suggests that variation in the ability to self-fertilise

occurs here too. Self-compatible trees may even have been inadvertently selected for introduction if they were more fecund than self-incompatible ones, due to a lack of pollen limitation of fruit set (Larson and Barrett, 2000).

High inbreeding depression, as found in *A. dealbata* in this study, is typical for trees (Barrett et al., 1996; Husband and Schemske, 1996; Duminil et al., 2009), including *Acacia*. Having fewer seeds per fruit after self- than cross-pollination has been documented for *A. dealbata* in this study (Fig. 2b) and for *Acacia baileyana*, *Acacia mearnsii*, *Acacia decurrens* and *Acacia melanoxylon* (Moffett and Nixon, 1974; Morgan et al., 2002, J.G. Rodger, unpublished results). This is most likely due to higher rates of abortion of self-fertilised progeny during development. Selfed progeny of *A. decurrens* and *A. mearnsii*, like *A. dealbata* (Fig. 3), also displayed inbreeding depression in growth and survival, segregating for lethal and less severe deleterious traits at the seedling stage as well as during later growth (Philp and Sherry, 1946; Moffett, 1956; Moffett and Nixon, 1974). The expression of characters associated with lower rates of survival was higher in selfed- than crossed-progeny in *A. dealbata* in this study. It is possible that these characters (Table S11), most of which seemed discrete rather than continuous, reflect the expression of particular deleterious recessive alleles in selfed progeny. However, as we were not able to determine the ratios of segregation, this could not be confirmed. Progeny were maintained to three months in this experiment, by which time substantial inbreeding depression in terms of survival from sowing ($\delta = 0.28$), and to a lesser extent growth ($\delta = 0.06$), was evident, and this would almost certainly have increased over time. This level of inbreeding depression to three months is comparable to that in the self-compatible trees *Eucalyptus regnans* (Hardner and Potts, 1997), *Fuchsia excorticata* and *Sophora microphylla* (Robertson et al., 2011), which all showed substantial inbreeding depression at later stages.

Habitually selfing plants generally experience lower levels of inbreeding depression (Husband and Schemske, 1996) as selfing exposes deleterious recessive alleles to selection (Crnokrak and Barrett, 2002). Self-compatible trees, however, often display high inbreeding depression, even if they have mixed mating systems or are predominantly selfing (Hardner and Potts, 1997; Ishida, 2006; Robertson et al., 2011). Theory indicates that when rate of mutation is high, as is characteristic of trees (Schofield and Schultz, 2006), there is a threshold in selfing rate below which genetic load cannot be removed. This is because virtually all selfed progeny will be homozygous for at least one lethal recessive so will fail to reach reproduction (Lande et al., 1994; Morgan, 2001). The high levels of both early and late inbreeding depression displayed in *A. dealbata* in this study, and those found in other self-compatible trees (Hardner and Potts, 1997; Ishida, 2006; Robertson et al., 2011) are consistent with this scenario. In *Magnolia obovata*, for instance, the primary selfing rate was over 0.8 but lifetime inbreeding depression was 0.97 (Ishida, 2006).

Despite the suggestion that selfing has little demographic relevance in trees because selfed progeny almost never reach maturity (Hardner and Potts, 1997; Ishida, 2006; Robertson et al., 2011), it is plausible that during invasion, sufficiently benign environmental conditions allow some selfed progeny to survive to reproduction. In the introduced range, where much suitable habitat is unoccupied, long distance dispersal would ensure that some selfed progeny would escape competition with outcrossed progeny. Selfing is also more likely to contribute positively to fitness when reproduction is precocious, because selfed progeny have to cope with the effects of inbreeding depression for only one or two years to survive to reproduction (Duminil et al., 2009). Precocious reproduction is associated with invasiveness in conifers (Richardson et al., 1994; Richardson and Rejmanek, 2004) and in *Acacia* (Gibson et al., 2011). Intriguingly, the Kapok tree *Ceiba pentandra* (Malvaceae), which colonised Africa by long distance dispersal from South America, is completely self-compatible in Africa (Baker, 1965b cited in Dick et al., 2007) but populations in South and Central America range from self-incompatible to at

least partially self-compatible (Lobo et al., 2005 and references therein). This suggests that selfing played a role in successful natural long distance colonisation for this species (Dick et al., 2007) and could also contribute to invasion in trees.

Thus despite high inbreeding depression, the possibility that selfing promotes invasion (or natural long distance colonisation) in trees cannot yet be rejected. This could be resolved by using genetic markers such as microsatellites to test whether selfed progeny of self-compatible trees such as *A. dealbata* do survive to reproduce during invasion.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.sajb.2013.08.002>.

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