Self-compatibility provides little reproductive assurance in the summer-flowering *Aloe reitzii* var. *reitzii*

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ABSTRACT

Floral adaptations, notably floral design and display, enable plants to govern their mating opportunities through pollinator attraction and pollen dispersal. However, large floral displays, together with multiple simultaneously open flowers in an hermaphroditic plant, increase the potential for geitonogamous self-pollination. Many angiosperms have evolved self-incompatibility that prevents self-fertilization, instead requiring outcrossing by pollen vectors for successful seed set. We investigated the reliance of the summer-flowering *Aloe reitzii* var. *reitzii* on pollinators for reproduction and by using controlled hand-pollination experiments to determine whether *Aloe reitzii* var. *reitzii* is self-compatible. Contrary to most aloes, *Aloe reitzii* var. *reitzii* is shown here to be partially self-compatible, but reliant on pollinators to transfer pollen between flowers within the same raceme or plant, as well as to other plants. *Aloe reitzii* var. *reitzii* appears incapable of autonomous self-pollination and pollinator-mediated geitonogamy causes reduced fecundity in *Aloe reitzii* var. *reitzii*. However, hand-selfed treatments produced viable seeds capable of germinating, suggesting that the partial self-compatibility in *Aloe reitzii* var. *reitzii* does provide some reproductive advantage and may have evolved due to its restricted distribution and increasing population fragmentation − possibly due to anthropogenic change.

1. Introduction

Angiosperms have evolved a diverse array of mating strategies due to distinctive features of their biology, such as immobility, modularity (clonal growth or large plant size) and hermaphroditism; together these add to the complexity of plant breeding systems (Barrett, 2003). The immobility of plants has resulted in the need for pollen vectors for mating between individuals (Barrett and Harder, 2017), promoting the evolution of diverse floral adaptations to attract specific biotic vectors for more effective cross-pollination (Harder and Barrett, 1996; Barrett, 2003). For example, various floral traits have evolved that are linked to specific pollinator guilds (Rosas-Guerrero et al., 2014), mainly through size variation and morphological complexity which facilitate or limit pollinator access to rewards, and the extensive diversification of these features in plants can be attributed to the need for successful mating (Harder and Barrett, 1996; Barrett, 2003; Harder and Johnson, 2005).

Floral adaptations that promote mating success, namely floral design (structure, color, scent and rewards; Barrett and Harder, 1996) and floral display (a combination of flower size, number of flowers, flower spacing and flower longevity; Willmer, 2011), enable plants to influence their mating opportunities through pollinator attraction

and pollen dispersal (Thompson, 2001; Barrett, 2013; Armbuster, 2014). Floral rewards promote mating success by ensuring continued visitation by pollen vectors (Barrett and Harder, 2017), and sufficient pollen removal by influencing the duration of pollinator visitation (e.g., through nectar production; Harder and Thomson, 1989). Outcross pollination largely depends on the types of pollinators visiting flowers and selection favours traits that influence pollinator behavior, to promote efficient pollination (Barrett and Harder, 1996; Barrett, 2003; Mitchell et al., 2009; Sletvold and Ågren, 2010; Barrett and Harder, 2017). Hence, morphological traits that characterize floral design and floral display influence the foraging behavior of pollen vectors to enhance fecundity (Harder and Barrett, 1996; Mitchell et al., 2009; Karron and Mitchell, 2012) and ensure efficient cross-pollen transfer by pollen vectors (Harder and Barrett, 1996). However, these floral rewards and attractants may also lead to pollination between flowers on the same individual (geitonogamous self-pollination; de Jong et al., 1993), especially in plants with large floral displays and many simultaneously receptive flowers (e.g., Snow et al., 1996; Galloway et al., 2002; Mitchell et al., 2004; Karron and Mitchell, 2012).

The fitness costs of geitonogamous self-pollination often result from reduced seed set in self-compatible plants, and from inbreeding depression in self-compatible plants (de Jong et al., 1993; Eckert, 2000). In contrast, autogamy (within-flower pollination;

Richards, 1997) can occur without pollinator visitation, and may therefore be advantageous as it can provide some reproductive assurance, especially when mates or pollen vectors are scarce (Eckert, 2000; Barrett, 2013). Additionally, there are no mating costs for autonomous self-pollination after outcrossing (delayed autogamy; Barrett and Harder, 2017). To limit selfing in hermaphroditic flowers, various anti-selfing mechanisms have evolved in plants, such as self-incompatibility (Seavey and Bawa, 1986), and spatial and/or temporal separation of male and female organs (herkogamy and dichogamy, respectively; Lloyd and Webb, 1986; Webb and Lloyd, 1986).

In addition, many angiosperms have evolved self-incompatibility mechanisms that prevent self-fertilization (Barrett, 1988), and reduce the costs of geitonogamy due to ovule discounting, i.e., loss of seed production when self-pollen disables ovules through early inbreeding or late-acting self-incompatibility (Duffy et al., 2013). Outcrossing by pollinators in self-incompatible plants (e.g., *Kniphofia linearifolia* Baker and *K. caulescens* Baker) ensures successful reproductive output (Duffy et al., 2013), thereby mitigating the costs of geitonogamy associated with large displays of simultaneously open bisexual flowers. However, pollen discounting (reduced amounts of cross-pollen reaching conspecifics due to self-pollination; Holsinger et al., 1984; de Jong et al., 1993; Richards, 1997; Barrett, 2002, 2003; Harder and Johnson, 2005) can still occur in self-incompatible plants, reducing male fitness as fewer seeds are sired (Harder and Barrett, 1995), while ovule discounting by self-pollen reduces female fitness (Duffy et al., 2013).

South Africa has the largest number of *Aloe* species of any African country (140 taxa; Klopper et al., 2009). Although the pollination biology and breeding system of aloes has been well investigated (e.g., Hoffman, 1988; Stokes and Yeaton, 1995; Botes et al., 2008, 2009; Hargreaves et al., 2012), the breeding system of some *Aloe* species has not yet been experimentally investigated (e.g., *A. peglerae* Schonl., Arena et al., 2013; Payne et al., 2016). As many aloes have large floral displays, they have evolved various anti-selfing mechanisms that may serve to reduce the incidence of geitonogamous self-pollination. These include late-acting (ovarian) self-incompatibility (Hargreaves, 2007; Hargreaves et al., 2012), protandry (Botes et al., 2008; Hargreaves et al., 2012), herkogamy (Hargreaves et al., 2012) and the acropetallous maturation of flowers on the raceme (Botes et al., 2008).

Self-incompatibility is common in the genus *Aloe* L. (Cousins et al., 2013), with most *Aloe* species studied to date found to be self-incompatible, including, *A. ferox* Mill. (Hoffman, 1988); *A. inconspicua* Plowes (Hargreaves et al., 2008); *A. marlothii* A.Berger (Symes and Nicolson, 2008); *A. linearifolia* A.Berger (Botes et al., 2009); and *A. pruinosa* Reynolds (Wilson et al., 2009). Self-compatibility has been observed in very few *Aloe* species (Ratsirarson, 1995; Pailler et al., 2002), with *A. thraskii* Baker the only South African aloe known to be self-compatible (Patrick et al., 2018). Self-incompatibility in aloes may enable them to maintain a high seed set through reduced ovule discounting by relying on cross-pollen transfer by pollen vectors for successful seed set (Duffy et al., 2014). Animal pollinators therefore play a crucial role in the fecundity of aloes; hence investigating breeding systems in *Aloe* is important in determining the diversity and success of plant-pollinator interactions (Arena et al., 2013). This knowledge may contribute to the management and conservation of wild *Aloe* populations and their respective environments (Cousins and Witkowski, 2012).

Similar to many *Aloe* species, *A. reitzii* Reynolds var. *reitzii* (hereafter referred to as *A. reitzii*) has floral traits consistent with an ornithophilous pollination syndrome (Van Wyk and Smith, 2008). Although birds and bees are abundant visitors to *A. reitzii* flowers (Symes, 2017; Payne et al., 2022), the aloe's reliance on cross-pollen transfer by animal vectors for reproduction has not yet been investigated. Therefore, we aimed to establish whether *A. reitzii* is dependent on pollen vectors for successful seed set by investigating its breeding system and whether, like many other aloes, it is self-incompatible. Controlled hand-pollination experiments were thus conducted to deduce whether *A. reitzii* is self-incompatible and its dependence on pollinators for reproduction.

2. Materials and method

2.1. Study species and study site

Aloe reitzii (Asphodelaceae) is a stemless aloe, reaching a height of up to 1 m (Symes, 2017), with a mean leaf rosette height of 66.59 cm, and a mean rosette diameter of 60.55 cm (Payne et al., 2022; Fig. 1A). It is characterised by dark red flowers that turn yellow as they mature (Van Wyk and Smith, 2008). Its distinctly curved, long (up to 50 mm), tubular, downward pointing flowers distinguish this Near-Threatened (Mtshali et al., 2018a) species from similar species (e.g., *A. aculeata* Pole-Evans, *A. gerstneri* Reynolds and *A. petricola* Pole-Evans; Van Wyk and Smith, 2008). On average, *A. reitzii* plants have three racemes, with up to ten racemes observed on a single plant (Symes, 2017).
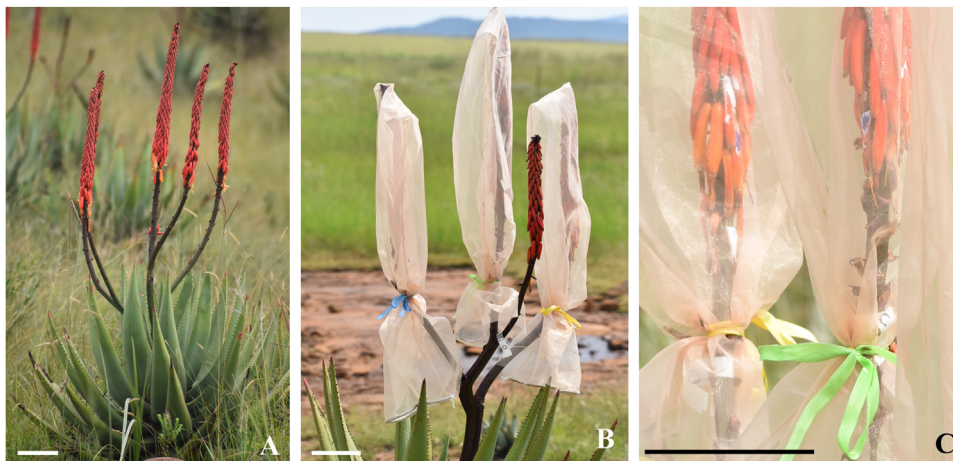

Fig. 1. (A) Mature *Aloe reitzii* var. *reitzii* plant with multiple racemes; (B) Autogamous, cross- and self-pollinated treatments of *A. reitzii* var. *reitzii* represented by blue, green and yellow ribbons, respectively, while open treatments (no bag present) allowed all floral visitors; (C) *Aloe reitzii* var. *reitzii* flowers showing the strongly exserted filaments; (D) Marked flowers in the self- and cross-pollination treatments used in the hand-pollination experiments. Scale bar = 10 cm. Photographs: Jessica Minnaar.

Contrary to most ornithophilous aloes, *Aloe reitzii* flowers in summer — between February and March (Van Wyk and Smith, 2008). It is endemic to the rocky slopes and grasslands in regions around Roossenekal and Belfast across to the Barberton Mountains in Mpumalanga, South Africa (Mtshali et al., 2018a), but localised distributions in Limpopo and Gauteng have also been noted (Van Wyk and Smith, 2008). The spring-flowering variety, *Aloe reitzii* Reynolds var. *vernalis* D.S.Hardy, is Vulnerable and is known from a single isolated population in northern KwaZulu-Natal (Mtshali et al., 2018b).

The study was conducted on Klipbankspruit Farm approximately 30 km north-east of Dullstroom, Mpumalanga, where *Aloe reitzii* grows in abundance. The privately-owned farm is used for commercial beef farming and grass baling (Symes, 2017; Payne et al., 2022).

2.2. Breeding system

The study took place during the peak of one flowering season in February 2020. To test the degree of self-compatibility in *A. reitzii* and its dependence on pollinators for reproduction, racemes on 15 randomly selected aloes, each with four racemes or more, were bagged before anthesis with fine organza mesh bags (1 × 1 mm) whilst in bud, and one of four treatments was applied randomly to each raceme, each marked with a different coloured ribbon tied around it (Fig. 1B): (1) autogamous, unmanipulated flowers; (2) open, flowers unmanipulated but receiving pollen from all floral visitors (control); (3) crossed, flowers receiving pollen from another plant individual, via hand-pollination (xenogamy; Richards, 1997); (4) selfed, flowers receiving pollen from a flower on the same raceme, via hand-pollination (geitonogamy). Hand-pollinated and autogamous treatments remained bagged for the entire flowering season to prevent pollinator access, except for when bags were briefly removed for hand-pollination. For hand-pollinated treatments, five flowers were randomly selected per raceme, resulting in a total of 75 flowers per treatment. To avoid contamination of stigmas with self-pollen, flowers used in hand-pollinated treatments were emasculated before anthesis (Wilson et al., 2009; Duffy et al., 2013). Selected flowers were marked using white correction fluid on the corollas and pedicels and then marked with blue ink once pollinated (Fig. 1C). Hand-pollinated pollen transfers were conducted by brushing dehisced anthers across receptive stigmas as soon as they had elongated and their surface expanded (Patrick et al., 2018). To compensate for flowers that fell off the raceme following hand-pollination (likely due to damage), new flowers were pollinated to ensure each raceme had five hand-pollinated flowers at the end of the flowering season.

In late March, fruit from all treatments were collected. The number of flowers on each raceme was determined by the number of pedicels present after flowers had fallen off (Symes et al., 2009). For the autogamous and open treatments, fruit set was calculated by dividing the number of fruit produced by the number of flowers per raceme (Payne et al., 2019). For hand-pollination treatments, fruit set was calculated by dividing the number of fruit produced from the hand-pollinated flowers by the number of hand-pollinated flowers per raceme. Seeds per fruit (seed set) was calculated as the mean number of seeds produced per fruit for each raceme (Payne et al., 2019). For autogamous and open treatments, seed set was determined from randomly selecting 10 fruits per raceme. Total seed production per plant was calculated as the product of seed set per raceme and the number of fruit produced per raceme (Arena et al., 2013; Payne et al., 2019). Seeds produced per flower was calculated by dividing the total seed production by the number of flowers produced per raceme (Payne et al., 2019). For hand-pollination treatments, the total seed production was divided by the number of hand-pollinated flowers. Plants with no seeds (i.e. where no fruit was produced) in any of the treatments were excluded from any seed set analyzes. One aloe produced no fruit for all treatments, except for hand-selfed treatments,

and was removed from all fruit and seed set analyzes. Differences in fruit and seed production were compared among treatments.

2.3. Germination trials and seed viability

To test for seed viability of open and autogamous treatments, five fruits were randomly selected from each treatment raceme and a maximum of 10 seeds were randomly selected per fruit, resulting in a maximum of 50 pooled seeds per treatment raceme (Payne et al., 2016). Only mature seeds (dark gray in color) were selected. Similarly, for hand-pollination experiments, seed viability tests were conducted by randomly selecting 10 seeds from each fruit, resulting in a maximum of 50 pooled seeds per raceme (where possible). For each raceme, 10 seeds were randomly selected from these 50 pooled seeds, resulting in a total of 150 seeds per treatment (N = 15 aloes/treatment). Seeds were placed into sterilized Petri dishes on top of two filter papers. To prevent fungal growth during seed germination, the filter papers were washed in 2% bleach, followed by a double rinse in distilled water (Arena et al., 2013). The seeds were covered with one filter paper and saturated with distilled water. The Petri dishes were kept in an environmental control chamber, with daytime temperatures of 25 °C and night-time of 15 °C (12 h day-night cycle; Arena et al., 2013; standard germination conditions, S_{GC}). Germinating seeds, identified by a 2 mm radicle protruding from the seeds, were counted every day for three weeks, and thereafter, three times a week for an additional three weeks (Arena et al., 2013; Payne et al., 2016).

Seeds that had not germinated were removed from the control chamber and empty seed coats were removed and recorded as "dead". To determine seed viability of the ungerminated seeds, any intact seeds were sliced in half to expose the embryo and saturated with a 1.0% 2,3,5-triphenyltetrazolium chloride (tetrazolium) solution overnight (Arena et al., 2013; Payne et al., 2016). Seeds with embryos that had turned pink when viewed the following day were considered "viable", while seeds with embryos that remained white were recorded as "non-viable" (ISTA, 2003). Total seed viability (%) was calculated as the sum of seeds that germinated and those shown to be viable via tetrazolium seed testing divided by the total number of seeds per treatment used in the seed germination trials (Payne et al., 2016).

Since many seeds did not successfully germinate under these standard conditions, seeds were also germinated in ambient conditions (room conditions, R_{GC}). For each treatment, 50 seeds were randomly selected from pooled seeds/raceme and germinated (as outlined above). The Petri dishes were kept in a relatively well-lit environment, at room temperature (∼17−25 °C; ∼13.5 h, 10.5 h day-night cycle).

2.4. Data analysis

A non-parametric Kruskal-Wallis test was used to test for differences among treatments in: i) fruit set, ii) seed set, iii) total seed production; iv) seeds per flower; v) percentage germination; vi) seed viability for ungerminated seeds (tetrazolium tests), and vii) total seed viability (Shapiro-Wilks Normality Test, $P < 0.05$). *Post-hoc* analysis was conducted on significant results using the Kruskal-Wallis Multiple Comparison *Post-hoc* Test (R package 'pgirmess'; Giraudoux et al., 2018). Statistical analyzes were performed in R (v. 4.0.0; R Core Team, 2020). Results are presented as mean ± standard error. Generalized linear models were not used to test for differences in fruit and seed analyzes because of the large number of zeros in each treatment (plants with no fruit, hence no seeds), especially in some unsuccessful hand-pollinations where no fruit was produced [cross-pollinations (N = 8) and self-pollinations (N = 8)].

3. Results

3.1. Breeding system

To test if *A. reitzii* is dependent on pollinators for reproduction and subsequent seed set, or if it can autonomously self-pollinate, open and autogamous treatments were used, and controlled hand-pollination experiments were conducted to determine whether *A. reitzii* is self-compatible. Fruit set differed significantly among treatments (H = 10.408, df = 3, $P < 0.05$), with selfed treatments (0.08 ± 0.03 fruits per raceme) producing significantly fewer fruits per raceme compared to open treatments (0.23 ± 0.05 fruits; $P < 0.05$; Fig. 2A). Seed set differed significantly among treatments (H = 13.62, df = 3, $P < 0.01$; Fig. 2B), with autogamous treatments (7.28 ± 2.57 seeds per fruit) producing significantly fewer seeds compared to crossed treatments (37.61 ± 5.54 seeds per fruit; $P < 0.05$). Total seed production differed significantly among treatments (H = 13.2, df = 3, $P < 0.01$; Fig. 2C), with autogamous (108.9 ± 27.52 seeds per raceme) and selfed treatments (31 ± 11.12 seeds per raceme) producing significantly fewer seeds compared to open treatments (1557 ± 373.86

seeds per raceme; $P < 0.05$). Seeds per flower differed significantly among treatments (H = 18.61, df = 3, $P < 0.001$; Fig. 2D), with autogamous treatments (0.37 ± 0.1 seeds per flower) producing significantly fewer seeds per flower compared to crossed (16.07 ± 4.2 seeds per flower; $P < 0.05$) and open (6.38 ± 1.48 seeds per flower; $P < 0.05$) treatments.

3.2. Germination trials and seed viability

Seed germination trials were conducted to infer seed viability of open and autogamous treatments, and the controlled hand-pollination treatments. In standard germination conditions (S_{GC}), seeds only began germinating after 20 days. Percentage germination was relatively low (except in crossed treatments), but highly variable across all treatments, ranging from 0 to 70% in crossed treatments, 0−30% in autogamous treatments, and 0−20% in open treatments. No seeds germinated in the selfed treatments. Percentage germination was highest in open ($13.0 \pm 2.13\%$; N = 100 seeds) and crossed ($12.86 \pm 9.93\%$; N = 62 seeds) treatments and was lowest in the
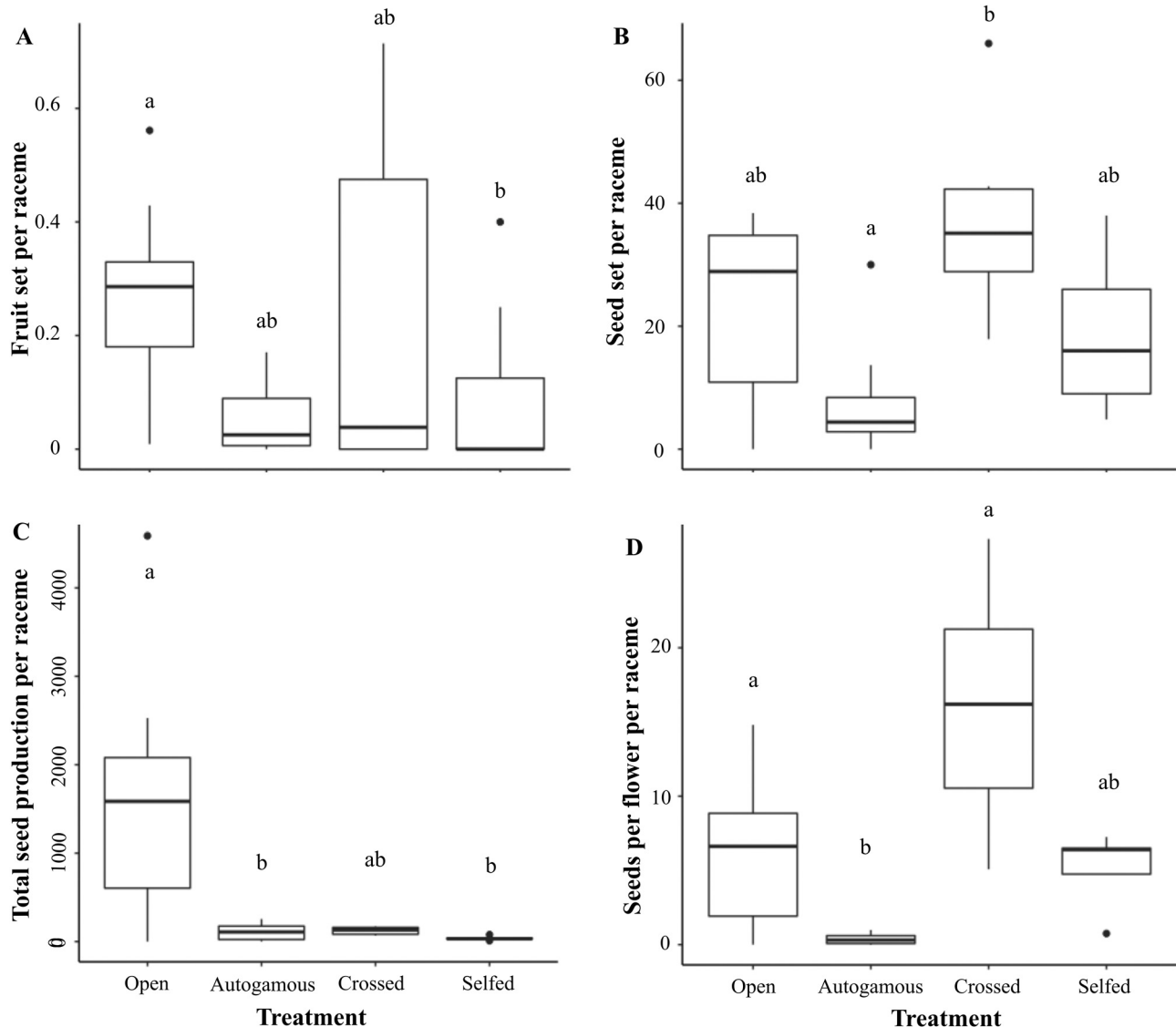

Fig. 2. Reproductive success of *Aloe reitzii* var. *reitzii* for open and autogamous treatments, and hand-pollination experiments during February to March 2020 flowering season (open, autogamous, crossed and selfed treatments), as measured by, (A) fruit set (proportion of flowers that set fruit) per raceme in all plants (N = 14) (B) Seed set (seeds produced per fruit) per raceme; (C) Total seed production per raceme; D) Seeds per flower per raceme. Boxes represent median and inter-quartile range, bars represent minimum and maximum, and solid circles represent outliers. Treatments with different letters are significantly different (Kruskal-Wallis Multiple Comparison, $P < 0.05$).
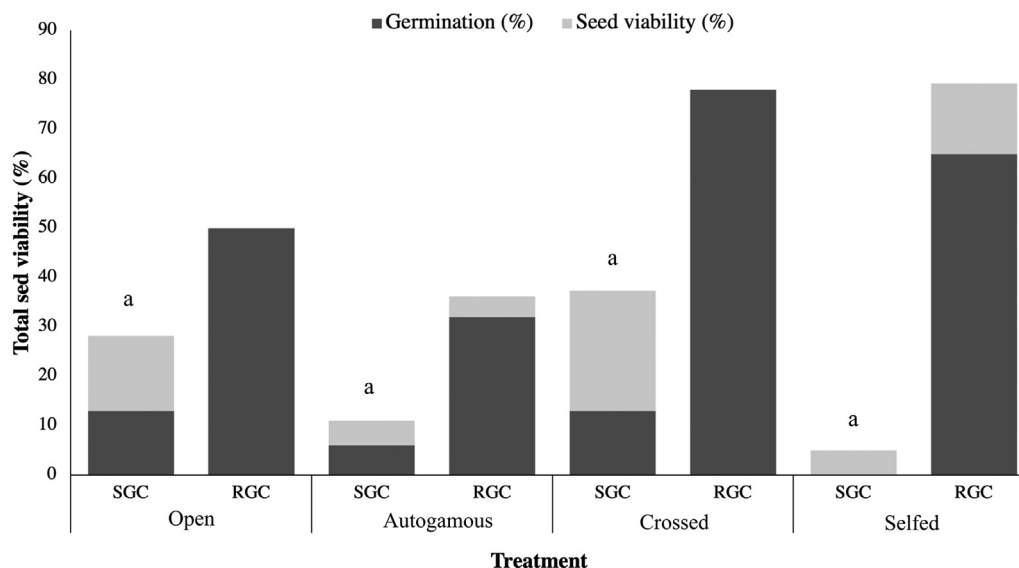
Fig. 3. Total seed viability (%), as shown by the sum of percent seed germination and percent viability of ungerminated seeds, for each treatment under standard germination conditions [S_{GC}; daytime temperature of 25 °C and night-time temperatures of 12 °C (12 h day-night cycle)] and ambient germination conditions (R_{GC}; ~17−25 °C; ~13.5 h, 10.5 h day-night cycle). Only mature seeds were used for germination trials and seed viability tests.

autogamous treatment (6.03 ± 3.49%; *N* = 80 seeds; Fig. 3). Percentage germination differed significantly among treatments (H = 12.5, df = 3, $P < 0.01$), with significantly higher percentage germination in open treatments compared to selfed treatments ($P < 0.05$). Seed viability of the ungerminated seeds (tetrazolium testing) did not differ significantly among treatments (H = 2.31, df = 3, $P > 0.05$). Total seed viability (germinated and tetrazolium testing combined) did not differ significantly among treatments (H = 7.62, df = 3, $P > 0.05$). Coefficients of Variation (CV's) for total seed viability for open, autogamous, crossed and selfed treatments were 46%; 167%; 109%; 110%, respectively. The high CV's indicate a high dispersion around the mean, explaining the lack of statistical difference among treatments.

In the follow-up germination trial, a higher percentage of seeds germinated per treatment when seeds were placed in a relatively well sunlit environment (Room germination conditions (R_{GC}); Fig. 3) at ambient temperature (~17−25 °C). In this environment, seeds from all treatments germinated within four days. Percentage germination was highest in crossed (78%; *N* = 50 seeds) and selfed (65%; *N* = 38 seeds) treatments, and lowest in open (50%; *N* = 50 seeds) and autogamous (32%; *N* = 50 seeds) treatments (Fig. 3). Only a small percentage of the seeds that did not germinate were viable for the selfed (14.29%) and autogamous (4.17%) treatments, with none in the crossed and open treatments (Fig. 3), when tested using Tetrazolium salts.

4. Discussion

Our investigation into the reliance of *A. reitzii* on pollinators for reproduction shows that it is not capable of autonomous self-pollination; it is therefore dependent on pollinators for pollen transfer between flowers on the same plant and on different plants. This is shown by the lower seed set, total seed production, seeds per flower and low seed viability in the autogamous treatments compared to open and crossed treatments. Insects, namely thrips and aphids, can mediate self-pollination (Baker and Cruden, 1991), and thrips were common visitors to *A. reitzii* flowers and were often observed crawling around flowers of autogamous treatment racemes, along with a variety of small beetles (J. Minnaar, pers. obs.). The presence of these insects inside the bagged treatments may have resulted in geitonogamous selfing amongst the protandrous flowers, rather than

autonomous selfing. This could account for the very limited fruit set and seed set in the autogamous treatment racemes. Additionally, rainfall and strong winds resulted in pollen being brushed onto the sides of bags (J. Minnaar, pers. obs.), which could have pollinated flowers with receptive stigmas as they matured acropetally. Therefore, it is quite likely that any fruit set and seed set in autogamous treatments is a result of geitonogamous selfing, rather than autogamous selfing.

Based on the formation of a small percentage of viable seeds in the hand self-pollinated treatments, it appears that *A. reitzii* can self-pollinate to some extent with the aid of a pollen vector. Self-compatibility is rare in *Aloe*, known to occur in only a few *Aloe* species, such as *A. thraskii* (South Africa; Patrick et al., 2018) and two island endemics, *A. divaricata* Berger (Madagascar; Ratsirarson, 1995) and *A. mayottensis* A.Berger (Mayotte, Indian Ocean; Pailler et al., 2002). As in *A. reitzii*, these self-compatible aloes require pollinators to transfer pollen between flowers within a raceme and between racemes on a plant (Ratsirarson, 1995; Pailler et al., 2002; Patrick et al., 2018).

Unlike the self-compatible *A. thraskii* (Patrick et al., 2018), *A. reitzii* experienced reduced fecundity from self-pollen, likely transferred via geitonogamous self-pollination. Geitonogamous self-pollination can also result in pollen discounting, reducing outcross siring success which may lower plant fitness due to inbreeding depression (de Jong et al., 1993). However, the level of possible inbreeding depression resulting from self-pollen deposition in *A. reitzii* needs to be quantified by comparing genetic markers in seeds produced by selfed progeny and mature plants. The large floral display with many simultaneously open flowers on the same raceme or plant increases the probability of geitonogamous selfing in *A. reitzii* (Harder and Barrett, 1995). Although *A. reitzii* appears to lack self-incompatibility mechanisms which may reduce the costs of geitonogamy, it does reduce its occurrence through protandry and herkogamy (the stamens mature first and elongate further than style; J. Minnaar, pers. obs.), as well as through acropetallous maturation of flowers along the raceme (Botes et al., 2008; Hargreaves et al., 2012).

Pollinators can mediate self-pollination by foraging within flowers (facilitated selfing) or between flowers on the same plant (geitonogamy; Lloyd, 1992; Lloyd and Schoen, 1992). Exclusion experiments have shown that bees play a minor role in the pollination of *A. reitzii* when compared to bird pollinators (Minnaar, 2021). Bees likely mediate geitonogamous self-pollination, as they forage

between flowers on the same raceme and/or plant (J. Minnaar, pers. obs.). Although birds were often observed feeding between racemes on the same plant, they were more frequently observed feeding between racemes among different *A. reitzii* plants (J. Minnaar, unpubl. data.). Hence, self-pollen, likely transferred primarily by bees, combined with cross-pollen predominantly via birds, suggests a mixed mating system (i.e., a combination of cross- and self-pollen in the same individual; Goodwillie et al., 2005) in *A. reitzii*. Since hand-selfed treatments in *A. reitzii* did produce viable seeds that were able to germinate, this could suggest that a mixed-mating system in *A. reitzii* provides some potential for reproductive assurance, while the potential for removal of self-pollen by bees may enhance fecundity (Duffy et al., 2013) and thereby ameliorate the effects of its self-compatibility.

Both *A. reitzii* var. *reitzii* and *A. reitzii* var. *vernalis*, have restricted and/or localized distributions (Mtshali et al., 2018a, 2018b), with their habitats increasingly under threat due to mining, quarrying and development. At least 30% of *A. reitzii*'s habitat has already been irreversibly modified, with most habitat loss attributed to commercial timber plantations between Belfast, Dullstroom, Lydenberg and Barberton (Mtshali et al., 2018a). Self-compatibility in *A. thraskii* is suggested to have evolved to reduce mate limitation within its metapopulation (Patrick et al., 2018), and the other two aloes known to be self-compatible occur on islands, where a population bottleneck would have naturally occurred. Therefore, self-compatibility in *A. reitzii* may have arisen through changing population dynamics and plant-pollinator interactions to reduce mate limitation because of habitat fragmentation (Eckert et al., 2009), hence providing some reproductive assurance. This may be tested by establishing whether an increase in homozygosity has occurred over time in either one or both varieties of *A. reitzii* because of possible inbreeding depression. In addition, breeding trials should investigate whether *A. reitzii* var. *vernalis* is also self-compatible to any degree.

Seed germination and seed viability of *A. reitzii* were very low for both germination conditions compared to other seed germination studies on *Aloe* (e.g., Arena et al., 2013; Cousins et al., 2013; Payne et al., 2016; Kremer-Köhne, 2018). This may be explained by germinating *A. reitzii* seeds in the same growth conditions as winter-flowering aloes (e.g., *A. peglerae*, Arena et al., 2013, Payne et al., 2016; and *K. plicatilis*, Cousins et al., 2013), which would likely germinate in spring — in warmer temperatures with more frequent rainfall. Since *A. reitzii* fruits dehisce mid- to end-April, seeds are likely to germinate in cooler, autumn temperatures with less frequent rainfall, which could explain the higher percentage of seeds that germinated at cooler (∼17−25 °C) room temperatures.

5. Conclusion and future recommendations

Aloe reitzii is only the second South African aloe observed to be self-compatible (to any degree) and, like other known self-compatible aloes, *A. reitzii* appears to be reliant on pollinators for pollen transfer between flowers. Self-pollen reduces fecundity of *A. reitzii*, suggesting that self-compatibility in *A. reitzii* could result in inbreeding depression. However, an increase in homozygotes for detrimental alleles needs to be established to support this hypothesis. Pollinator-mediated geitonogamous selfing predominantly by bees in *A. reitzii* results in seed discounting and reduced fecundity. The effects of pollen discounting and ovule discounting resulting from self-pollen deposition should be quantified through floral emasculation (see Duffy et al., 2013), and examination of pollen tube growth rates in selfed and crossed individuals. Self-compatibility may have evolved due to the aloe's restricted distribution, and further investigation of the breeding system of *A. reitzii* var. *vernalis* could provide insight into the population dynamics of both *A. reitzii* varieties that may have resulted in the evolution of self-compatibility and/or self-incompatibility in these taxa with restricted distributions.

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