Reproductive ecology of *Aloe plicatilis*, a fynbos tree aloe endemic to the Cape Winelands, South Africa

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**ABSTRACT**

While the pollination ecology of many *Aloe* species is well-documented, knowledge on aloe seed ecology, and hence aloe reproductive ecology in its entirety is limited. The aim of this study was to investigate the reproductive ecology of *Aloe plicatilis*, a Cape fynbos tree aloe endemic to the Cape Winelands, South Africa. Results from a pollinator exclusion experiment conducted at an *A. plicatilis* population on Paarl mountain suggests pollination primarily by insects, although bird visitation significantly increased seed set/fruit indicating possible co-pollination with insects. The species’ long-tubed flowers and production of concentrated nectar, with observations of malachite sunbirds as the most common avian visitors to *A. plicatilis* flowers indicate the importance of long-billed specialist avian nectarivores as floral visitors. Analysis of the relationship between plant size and inflorescence production for five populations combined revealed a significant, positive linear relationship between plant size and the logarithm of the number of inflorescences/plant. Natural fruit and seed set determined for three populations (1325, 27,930 and 251,616 seeds/population) suggests low reproductive output compared to several other *Aloe* species. The smallest (31 individuals) and least dense (75 plants/ha) *A. plicatilis* population produced the lowest seed set/plant (128 seeds) and per population (1325 seeds), suggesting an Allee effect. Evaluation of seed dispersal potential showed that potential dispersal distances were approximately three times the canopy height; however, the occurrence of *A. plicatilis* on mountains isolated from more continuous mountain ranges on which the species also occurs suggests the possibility of long-distance dispersal by strong, gusty, summer winds. Soil seed bank samples collected from 13 populations yielded close to zero seedling emergence, indicating the absence of persistent seed banks. *A. plicatilis* seeds stored under ambient laboratory conditions for 3, 18 and 24 months were germinated in an environmental control chamber and a laboratory. High percentage germination was recorded for 18- and 24-month-seed (86 and 80%, respectively), while germination of 3-month-old seeds was three times lower, suggesting the need for after-ripening. Germination of fresh and one-year-old seed under ambient nursery conditions at the Karoo Desert National Botanical Garden in Worcester yielded emergence percentages of 67 and 44%, respectively, and were therefore less successful than germination under more controlled conditions. This is the first known study to investigate the reproductive ecology of a tree aloe species and that of a Cape fynbos aloe. The study highlights the importance of further studies on aloe seed ecology, particularly for rare and threatened species.

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**1. Introduction**

Knowledge of plant reproductive ecology is key to understanding many community processes such as regeneration, establishment, succession and alien species invasion, as well as species survival strategies and causes of rarity (Grubb, 1977; Bazzaz, 1979; D’Antonio, 1990; Gutierrez, 1994; García-Fayos and Verdú, 1998; Kaye, 1999). Pollination, which is an integral part of plant reproductive ecology, deals with pollination strategies, flowering phenology and patterns of nectar, fruit and seed production (Lovett Doust and Lovett Doust, 1988), while seed ecology covers dispersal, dormancy and germination (Fenner and Thompson, 2006). The persistence of a plant population is a product of its constituent individuals’ collective reproductive success which, in turn, is affected by factors such as plant morphology (size, shape and positioning of plant parts), competition, herbivory, population size and density, and the availability of pollen for fertilization and resources for seed production (Lovett Doust and Lovett Doust, 1988; Stephens et al., 1999; Mustadžarvi et al., 2001; Wilcock and Neiland, 2002).

Each species possesses a suite (or syndrome) of floral traits that suggest adaption to a particular mode of pollination, either self- or cross-pollination (Fägri and van der Pijl, 1979; Primack, 1987; Dafni, 1992; Fenster et al., 2004). Descriptions of syndromes emphasize...
characteristics such as flower colour, odour, size, shape, rewards, and timing of anthesis (Primack, 1987; Dafni, 1992). Flowers pollinated by energy-demanding vertebrates such as birds and bats are generally large and produce copious amounts of food rewards, while flowers visited by small insects tend to be smaller and produce less food rewards (Primack, 1987). Individual plant species are associated with a particular pollination system that occurs along a continuum from generalized (hundreds of pollinator species) to extremely specialized (a single pollinator species) (Johnson and Steiner, 2000). Most animal-pollinated plants exhibit moderate to highly generalized pollination systems (Waser et al., 1996); however, there is a growing body of literature documenting very specialized pollination systems in the tropics and in the species-rich temperate flora of South Africa (see citations in Johnson and Steiner, 2000). Although floral syndromes may provide clues about potential pollinators, they do not necessarily provide a foolproof means of predicting all a species' floral visitors, and visitors that do not match the species' floral syndrome may still be important and should not be overlooked (De Merxen et al., 2009).

Seeds play four important roles in the persistence of species, viz. reproduction, dispersal within the same plant community, expansion into new habitats, and survival of germplasm through seasons or environmental conditions unfavourable for growth (Vásquez-Yanes and Orozco-Segovia, 1993). Fruit and seed set, followed by seed dispersal are critical determinants of a plant's reproductive success and may influence the distribution and size of populations (Steffan-Dewenter et al., 2012). Seed set is dependent on interactions with pollinators, seed predation, nutrient availability and microclimatic site conditions (Steffan-Dewenter et al., 2012). Seed dispersal affects plant population dynamics by reducing density-dependent mortality near parent plants, expanding a species' range on a regional scale, and shaping population genetic structure (Wiebel and Thomson, 1995; Corlett, 2009; Howe and Miriti, 2004). While short-distance dispersal shapes the dynamics of local populations and communities, long-distance dispersal is central to the large-scale dynamics thereof (Schurr et al., 2009). Dispersal distances are often difficult to determine in the field, hence rate of seed descent can be used as an index of dispersal capability, which has often been related to seed size and mass (see citations in Matlack, 1987).

Sometimes dispersed seeds germinate immediately, but in most cases there is a delay, which is brought about either by quiescence or dormancy (Fenner and Thompson, 2006). Wild plant populations often form stores of dormant or quiescent seeds in seed banks, which may last for long periods of time, with intermittent germination of part of the seed bank (Murdoch and Ellis, 2000). Dormancy is caused by a block to germination within an imbibed seed (Murdoch and Ellis, 2000). Thus, dormancy is a temporary failure of a viable seed to germinate after a specified period of time under a particular set of environmental conditions (e.g. moisture availability and temperature) that later induce germination when the restricted state has been terminated by either natural or artificial means (Vleeshouwers et al., 1995). Quiescent seeds, on the other hand, are those that remain ungerminated because the environmental conditions favourable for radicle emergence and seedling growth are lacking (Murdoch and Ellis, 2000). Seed banks comprising dormant or quiescent seeds may be transient (persisting in the soil for 1–5 years) or long-term persistent (>5 years) (Bakker et al., 1996). Following this inactive state, the successful germination and survival of a proportion of seedlings is crucial for the persistence of plant populations, and underpins the development and sustainability of plant communities (Murdoch and Ellis, 2000; Leck et al., 2008).

The Allee effect is a phenomenon of prime importance when investigating reproductive ecology in plant populations, particularly in studies on species with small, sparse and/or fragmented populations and which rely on animal vectors for pollination (Stephens et al., 1999). The Allee effect refers to a positive relationship between any component of individual fitness (e.g. probability of reproducing or dying) and either numbers or density of conspecifics (Stephens et al., 1999; Berec et al., 2007). Decreases in the fitness of individuals often occur in small and/or sparse populations as population size and/or density decline (Berec et al., 2007; Kramer et al., 2009). Plants in such populations tend to receive fewer visits from pollinators, which may not only result in lower seed set (e.g. Lamont et al., 1993a), but also inbreeding depression (e.g. Groom, 1998). If populations drop below the Allee threshold – the size or density at which population growth rate becomes negative (Berec et al., 2007) – this can have dire consequences for the persistence of rare and threatened species.

Aloe L., is the largest genus in the Asphodelaceae, a family of succulent-leaved, petaloid monocots (Smith and Van Wyk, 2009), found primarily on the African continent (see distribution map in Cousins and Witkowski, 2012). With ±140 Aloe species, South Africa has the highest aloe diversity of any African country (Klopper et al., 2009). Aloes produce tubular flowers on many-flowered spikes, simple racemes, or compound racemes, which tend to be densely flowered and brightly coloured (Smith and Van Wyk, 2009). Aloes are generally self-incompatible and therefore reliant on animal floral visitors for pollination and seed set (Hoffman, 1988; Botes et al., 2009). Many Aloe species attract insect and bird pollinators by supplying abundant nectar primarily during winter, when alternative food sources are scarce (Beyleved, 1973; Nicolson and Nepi, 2005; Botes et al., 2008; Symes and Nicolson, 2008; Forbes et al., 2009; Symes et al., 2011). Studies on several Aloe species have confirmed the existence of extensive bird pollination systems in the genus (e.g. Hoffman, 1988; Ratsirarson, 1995; Johnson et al., 2006; Forbes et al., 2009; Botes et al., 2008; Symes and Nicolson, 2008; Arena et al., 2013). Avian pollinators are either specialist or opportunistic nectarivores, which may be filtered by specific floral traits, especially nectar properties (Johnson and Nicolson, 2008). Botes et al. (2008) showed that aloes that produce long-tubed flowers with small quantities of comparatively concentrated nectar are associated with specialist long-billed sunbirds as pollinators, while those producing short-tubed flowers that yield large volumes of relatively dilute nectar are generally pollinated by short-billed occasional nectarivores.

While the majority of Aloe species have floral adaptations consistent with bird pollination, insects are also frequent visitors to some of these species, usually nectar- and/or pollen-collecting bees (Hoffman, 1988; Hargreaves et al., 2008; Human and Nicolson, 2008; Botes et al., 2009; Symes et al., 2009; Wilson et al., 2009). However, Aloe species appear to show extensive variation in the contribution that bees make to pollination (Wilson et al., 2009). They may be exclusive pollinators (e.g. Hargreaves et al., 2008), co-pollinators with birds (e.g. Human and Nicolson, 2008; Symes et al., 2009), or resource robbers that contribute very little towards, or compromise, pollination success (e.g. Hargreaves et al., 2010). In some cases where bees co-pollinate with birds, the bees' contribution to reproductive success may equal or exceed that of the birds' (e.g. Symes et al., 2009).

Aloe seeds are typically 3–5 mm long, two-winged, smooth and triangular–elliptical in shape (Kamstra, 1971). They are wind-dispersed and in some Aloe species they possess a third wing, which may increase travelling distances (Jordan, 1996). The seeds of other Aloe species lacks wings, probably resulting in poor dispersal, and hence the establishment of dense seedling stands close to parent plants (Jordan, 1996, 1999). Aloe seeds are borne in fruits which are usually three-angled oblong dehiscent capsules (Kamstra, 1971). Despite aloes producing an abundance of seeds, establishment of recruits is infrequent and appears to be dependent on rainfall, which is often scarce and erratic across much of the range of the genus (Wabuyele and Kyalo, 2008). Aloe seeds typically germinate within three weeks of dispersal and their viability is often significantly reduced after a year (Giddy, 1973; Van Jaarsveld, 1989). The germination and establishment of aloe seedlings is dependent on nurse plants, which protect against excessive heat, solar radiation, desiccation, frost and herbivory (Giddy, 1973; Smith and Van Wyk, 2009).
Studies on aloe reproductive ecology have focused primarily on pollination and to a much lesser degree on seed ecology (Smith and Correia, 1992; Symes, 2012; Arena et al., 2013). Fewer still, have addressed aloe reproductive ecology in its entirety, from pollination through to germination. Hence, there is a paucity of information on several aspects of aloe seed ecology, including dispersal, dormancy, longevity, seed banks, and germination. The aim of this study was to investigate the reproductive ecology of Aloe plicatilis (L.) Mill., a fynbos tree aloe endemic to the Cape Winelands, South Africa. The objectives were to (a) determine the primary pollinator guild of the species, (b) quantify natural fruit and seed set, (c) conduct tests for the presence of seed banks, (d) investigate seed dispersal potential, and (e) conduct germination studies on fresh and stored seeds.

2. Materials and methods

2.1. Study species and area

A. plicatilis (L.) Mill. (Asphodelaceae: Alooideae) is one of six tree aloes indigenous to South Africa and the only tree aloe that occurs in the Cape fynbos (Van Wyk and Smith, 2008). The species has a restricted distribution in the mountainous parts of the Cape Winelands (also known as the “Boland”) in the south-western Cape (Fig. 1). The region is characterized by a Mediterranean climate with hot, dry summers (average midsummer temperatures = 15–25 °C) and cool, wet winters (average midwinter temperatures = 7–15 °C) (Manning, 2007). A. plicatilis grows in well-drained, acidic soils on steep rocky slopes and outcrops that offer protection from fires (Van Jaarsveld, 1989; Carter et al., 2011). The Cape fynbos has a crown fire regime, with fires occurring primarily during summer, at intervals of 10–30 years depending on the vegetation type (Keeler et al., 2012). A. plicatilis occurs at altitudes of 200–950 m, with average monthly wind speeds of 4.5–10.4 km/h and average annual rainfall and temperatures of 420–1900 mm/year and 14–18 °C, respectively (Cousins et al., submitted). A. plicatilis is relatively long-lived and slow-growing, with dichotomously branching stems, each of which ends in a set of 12–16 alternate, succulent leaves in a fan-like arrangement (Van Wyk and Smith, 2008; Carter et al., 2011) (Fig. 2c,d). Most A. plicatilis individuals reach reproductive maturity at ~15 cm stem diameter and ~0.8 m in height (Cousins et al., submitted). Adult plants average ~1.5 m in height, but exceptionally large individuals may reach up to 5 m (Van Wyk and Smith, 2008).

The flowers are simple, cylindrical and slightly acuminate, ±5 cm long and scarlet in colour, arranged in laxly flowered racemes (25–30 tubular flowers/raceme) that are 15–25 cm long (Reynolds, 1969) (Fig. 2a,f). A. plicatilis flowers from August–October (occasionally November) and the fruiting season starts in early November, with fruit capsules dehiscing from December to January (Van Wyk and Smith, 2008). The fruits are longitudinally-dehiscent capsules, approximately 20 mm long and 16 mm in diameter (Killick, 1988) (Fig. 2d). They are green with a pinkish tinge when fresh and pale manila-coloured when dehiscent (Killick, 1988). The winged seeds are dark brown to black (Killick, 1988).

2.2. Pollination ecology

A pollinator exclusion experiment was conducted at an A. plicatilis population on Paarl Mountain (W1, Figs. 1 & 2b) in order to determine whether the species is predominantly bird- or insect-pollinated. Three treatments were applied to three different inflorescences on 17 plants: total exclusion, partial exclusion and a control (Fig. 2b). Exclusion of all potential visitors (i.e. birds, insects and mammals) was achieved by placing a fine gauze bag (~0.25 mm mesh shade cloth) over each inflorescence and the leaf cluster from which it emerged. For the partial exclusion treatment, a cage made of rigid ±2 cm gauge wire mesh was placed around the inflorescences, allowing unrestricted access by bees and other insects, but excluding birds and small mammals. The control involved marking one inflorescence with identifying tape on each individual sampled, allowing unrestricted access by all floral visitors. The experiment commenced at the onset of the flowering season in August 2010 when flowers were at the bud stage so as to avoid any temporal bias. The plants were revisited during the fruiting season in December 2010 to collect the fruits and determine fruit and seed set. Fruit set was calculated as the percentage of pedicels on each inflorescence that bore fruit (which included both visible remains of pedicels and evidence of pedicels as scars on the raceme where flowers were attached). Seed set was determined per inflorescence and per fruit by counting the number of seeds per fruit for all fruits present on the inflorescence.

Four Bushnell® 8 mega pixel camera traps were set up for observations of animal visitors at the A. plicatilis population on the Paardeberg (PB; Fig. 1) for one week from 5 to 12 November 2012 during the peak flowering period. Each camera was positioned so as to encompass an entire A. plicatilis individual, but where possible, clumps of aloes were included in order to maximize the likelihood of observing floral visitors.

Standing crop nectar concentration was measured at two populations of A. plicatilis using an Eclipse® handheld refractometer. Measurements were taken mid–late morning (10 h00–12 h00) at W1 on 8 October 2011 and at PB on 29 October 2012. At W1, four flowering plants were selected, and for each plant two flowers on two different inflorescences were sampled, giving four flowers/plant and a total of 16 flowers. At PB, 10 individual flowering plants were selected, and 2–3 flowers from one inflorescence on each plant were sampled, giving a total of 26 flowers. Flowers were selected at a stage in their development corresponding with the flower indicated with an arrow in Fig. 2f. Observations of insect visitors at the inflorescences sampled for nectar concentration were recorded.

2.3. Relationship between plant size and inflorescence production

The relationship between plant size (stem diameter and height) and inflorescence production was determined from five A. plicatilis populations where data on flowering were available (DKK, DKM, GB, GS and V1) (see Fig. 1 for key to all population abbreviations). For all populations, individuals were sampled along transects using the Point Centred Quarter Method (Cottam and Curtis, 1956), with points spaced at 15–20 m intervals, except for V1 where all individuals were measured due to small population size (n ~ 48 individuals). Stem diameter was measured 10 cm above ground level using tree calipers and stem height (at the point of leaf emergence on the tallest stem) with a tape measure. The number of inflorescences was counted on each flowering individual, and the mean height of flowering individuals was calculated for three populations, viz. GB, JH and W1.

2.4. Natural fruit and seed set

Natural fruit and seed set were determined for GB, JH and W1. At each population, one inflorescence from ≥ 13 individuals was randomly selected, and for each inflorescence the following were counted: (a) the number of pedicels present (indicative of the number of flowers produced/inflorescence), (b) number of fruits/inflorescence, and (c) the number of seeds within each fruit. Fruit set/inflorescence was calculated as the percentage of flowers that produced fruit. Seed set was calculated as the mean number of seeds/fruit and mean number of seeds/inflorescence. Mean seed set/inflorescence was used together with mean fruit set/inflorescence and number of inflorescences/plant to estimate total fruit and seed production/plant. Total seed production/population was calculated by multiplying the percentage of flowering plants in each population by the average seed production/plant and the estimated population size.
2.5 Seed dispersal potential

Fresh *Aloe plicatilis* seeds were collected from PB in January 2012 to investigate seed dispersal potential. In May 2012, thirty seeds were weighed and their terminal velocity determined as an index of air lift for ease of dispersal using the method in Lamont et al. (1993b). The seeds were dropped individually from a height of 3 m in still air, while two observers measured the time taken for each seed to reach the ground using a stopwatch accurate to 0.01 s. Data on wind speed and direction at seven stations within the distribution of *A. plicatilis* were obtained from the Agricultural Research Council of South Africa. Potential dispersal distances were calculated for *A. plicatilis* individuals of three different sizes (a) the mean height at which the onset of reproductive maturity occurs i.e. 0.8 m, (b) the maximum recorded height for *A. plicatilis*, i.e. 4.0 m (Cousins et al., submitted) and (c) the mean height of flowering individuals.
calculated using height measurements and flowering data from V1, DKK, DKM, GS and GB. Dispersal distances were estimated using the following formula from Stokes and Yeaton (1995):

\[ d = \frac{1}{V_t} (h V_w) \]

where \( d \) = potential dispersal distance from an individual aloe in metres, \( V_t \) = terminal velocity of 2.7 m/s (calculated for \( A. plicatilis \) using the method in Lamont et al., 1993b), \( h \) = plant height (m), and \( V_w \) = average wind speed (m/s) at each wind station over the month of December, when seed dispersal is at its peak.

2.6. Soil seed banks

The presence of soil seed banks was determined on two separate occasions for a total of 13 populations. The first set of soil samples was collected from four populations (DKK, DKM, JH and W2) during the dry season in December 2010 – one year post-dispersal in 2009 and shortly pre-dispersal in 2010. The second set was collected from nine populations (AK, BK, GB, LM, SB, TK, TWK, V2 and ZH) during the rainy season in June 2011 – six months after seed dispersal in 2010. Samples were collected from beneath the canopies of five large reproductively mature individuals at each population. The soil was placed in seedling trays in the greenhouse at the University of the Witwatersrand, Johannesburg, within two to three weeks of collection. Shade cloth (30%) was placed over the glass roof of the greenhouse and plants were watered to saturation using automatic watering set at approximately 10 min once a day. Emergence of \( A. plicatilis \) seedlings was observed once a week over a period of six months.

2.7. Germination experiments

2.7.1. Germination under controlled and ambient laboratory conditions

A sample of fresh \( A. plicatilis \) seeds was collected from 2–3 inflorescences on ~40 plants at KK in December 2010. A second sample was collected in the same month ad hoc from three inflorescences on a single plant from each of the DKK, DKM and JH populations, which were also used as voucher specimens. The seeds from these three populations were pooled and used with the separate batch of seeds from KK to conduct seed viability tests after three different storage durations. The seed lots were stored in brown paper bags under ambient conditions in a cool, dry place, out of direct sunlight in a laboratory at the University of the Witwatersrand, Johannesburg.
The water content of a subset of 100 seeds from KK was determined on a fresh mass basis in the first week of March 2011. Seeds were weighed on a Precisa 92SM-202A scale correct to four decimal places, and then reweighed after oven-drying at 80 °C for 24 h (a temperature and duration similar to those used for determining the water content of other orthodox seeds e.g. Hay and Probert (1995) and Farrant and Walters (1998)).

Seed viability after three months storage was determined by germinating n = 300 seeds from KK in March 2012. Germination, which was defined as emergence of the radicle, was monitored once a week. The seeds were germinated in an environmental control chamber set at a light/dark cycle of 12 h/12 h, with temperatures at a constant 25 °C during the day and 15 °C at night, and a constant relative humidity of 50%. Light was supplied by fluorescent bulbs at ~650 nm. Seeds were placed in 90 mm diameter petri dishes, 25/dish. The 18-month-old seeds (n = 60 seeds; 30/dish) were germinated under ambient conditions on an east-facing laboratory windowsill in June 2012, since the environmental control chamber was not available. The 24-month-old seeds (n = 75; 25/dish) were germinated in December 2012 under the same conditions in the environmental control chamber described for the 3-month-old seeds.

For all three seed lots, the seeds were placed on two sheets of filter paper, covered by a third sheet and supplied with ±10 ml distilled water (or until the filter paper was saturated). The filter paper and distilled water were sterilized in an autoclave prior to use. Watering took place every 3–4 days; existing water was left in each dish and supplemented with fresh water until the filter paper was re-saturated. The seeds were inspected for germination once a week until cumulative germination was fairly constant (i.e. ≤2 new seeds germinating per week for ≥2 weeks). Accordingly, the 3-, 18-, and 24-month-old seeds were monitored over six, three and seven weeks, respectively. Germinated seeds were removed and planted in seedling trays to be grown for ex situ conservation in botanical gardens and private collections.

At the end of each experiment, standard tetrazolium tests were used to determine the viability of ungerminated seeds (Savonen, 1999). The seeds were sliced in half, keeping only one-half, which was placed in a 1% 2, 3, 5-Triphenyl-tetrazoliumchloride solution and stored under ambient, dark conditions. After 24 h the seeds were used to determine the viability of ungerminated seeds (Savonen, ex situ be grown for ≥1 week for germination was fairly constant (i.e. (\(q_i\)) = 0.003), with the all visitors’ and ‘insects only’ treatments both being significantly higher than the ‘no visitors’ treatment (Fig. 3b). Seed set/inflorescence followed the same pattern as fruit set (\(F_{2,46} = 6.77, p = 0.003\)), with the ‘all visitors’ and ‘insects only’ treatments being both significantly higher than the ‘no visitors’ treatment (Fig. 3c).

3. Results

3.1. Pollination ecology

Fruit set/inflorescence differed between treatments (\(F_{2,51} = 8.46, p = 0.0007\)), being significantly lower for inflorescences where all visitors were excluded. Exclusion of birds and mammals did not affect fruit set/inflorescence (Fig. 3a). Seed set/inflorescence followed the same pattern as fruit set (\(F_{2,46} = 7.77, p = 0.003\)), with the ‘all visitors’ and ‘insects only’ treatments both being significantly higher than the ‘no visitors’ treatment (Fig. 3b). Seed set/infl was also assessed for two different A. plicatilis flowers at W1. Mean standing crop nectar concentration at PB was significantly higher than at W1 (PB: 30.8 ± 7.91%, range = 21.0–46.0%; W1: 15.8 ± 3.1%, range = 9.5–21.5%) (\(t_{41} = 7.45; p < 0.0001\)). Overall nectar concentration for PB and W1 combined was 28.1 ± 12.0%.

3.2. Relationship between plant size and inflorescence production

There was a significant positive linear relationship between plant size and the logarithm of the number of inflorescences, with larger plants generally producing more inflorescences than smaller plants, however the relationship was stronger for stem diameter than for height (\(r^2 = 0.56\) and 0.45, respectively) (Fig. 4).

3.3. Natural fruit and seed set

There were no significant differences in fruit set/inflorescence between populations (17% at both W1 and GB and 13% at JH) (Table 1). Mean number of seeds/fruit ranged from 7 to 15. Total seed production/inflorescence ranged from 16 at JH to 50 at GB. Flowering...
plants were significantly taller at JH than at GB and W1. Mean number of inflorescences/individual ranged from 4 at GB to 8 at JH. W1 showed the highest calculated seed production/population due to its large estimated population size (3527 plants) and high seed production/individual. Calculated seed production/population at JH was lowest because of its small population size (31 plants) and low seed production/individual despite the larger plants (Table 1).

3.4. Seed dispersal potential

The seeds used for the terminal velocity test had a mass of 6.8 ± 1.0 mg and terminal velocity of 2.70 ± 0.41 m/s. Potential dispersal distances increased linearly with plant height (r² = 0.86; p < 0.0001), ranging from 1.3 m for 0.8 m individuals at LM near Franschhoek where the average monthly wind speed was lowest (4.5 ± 1.0 km/h), to 15.3 m for 4 m individuals in populations near Paarl (W1 and W2) where the average monthly wind speed was highest 10.4 ± 0.9 km/h (Table 2).

3.5. Soil seed banks

Soil samples collected a year after seed dispersal yielded no seedlings, suggesting the absence of a persistent seed bank. The second sample set collected six months post-dispersal yielded only four seedlings — two from ZH and one each from GB and SB, suggesting the presence of only a transient seed bank.

3.6. Germination

Seed water content prior to storage was 6.91 ± 1.83%. Mean weeks to germination for 3-, 18-, and 24-month-old seeds were 0.8, 2.5 and 2.3 weeks, respectively; hence, 3-month-old seeds germinated approximately three times faster than 18- and 24-month-old seeds. Total germination of 3-month-old seeds after six weeks (28%) was approximately one-third that of the 18- and 24-month-old seeds (86% after three weeks and 80% after seven weeks, respectively) (Fig. 5). Nevertheless, the tetrazolium tests showed that 100% of the ungerminated 3-month-old seeds were viable. The percentage of non-viable, empty seeds was similarly low for all three treatments (±11%).

Seeds sown in trays under nursery conditions emerged erratically up to six months after sowing. There was no association between percentage emergence of fresh and one-year-old seed and germination medium (χ² = 0.104; p > 0.90) (Table 3). Higher emergence percentages were obtained using fresh seed for all soil media compared with one-year-old seed, and fresh seed also yielded the highest average emergence across the three soil media (67%). Highest emergence/treatment was achieved using fresh seed in coarse river sand and soil from KK (both 72%).

4. Discussion

4.1. Pollination ecology

The pollinator exclusion results suggest that A. plicatilis is predominantly pollinated by insects. Differences in fruit and seed set/inflorescence were not significant between the ‘all visitors’ and ‘insects only’ treatments, which suggests that the contribution of birds to reproductive success is minimal. The relative abundance of honeybees (Apis mellifera) observed at GB and the general scarcity of birds at GB, PB

Fig. 3. Fruit and seed set (F ± S.E.) from the pollinator exclusion experiment at the Aloe plicatilis population at W1, south-western Cape, South Africa. The three treatments used were: (1) complete exclusion (‘no visitors’), (2) exclusion of birds and mammals (‘insects only’) and no exclusion (‘all visitors’) (n = 17 plants for each treatment), (a) Percentage fruit set per sampled inflorescence per plant, (b) seed set (number of seeds) per inflorescence and (c) seed set per fruit. Different letters indicate significant differences between treatments (Tukey HSD, p < 0.05).
and W1 would appear to support this interpretation. Other insects such as the two beetle species observed at W1 may also supplement pollination in A. plicatilis. Symes et al. (2009) showed that honeybees were largely responsible for the pollination of Aloe greatheadii var. davyana Schönland, and Wilson et al. (2009) confirmed the importance of insects in the pollination of Aloe pruinosa Reynolds. A. greatheadii var. davyana and A. pruinosa were also visited by sunbirds, which would be expected due to their low nectar volumes and high sugar concentrations compared to other Aloe species (14.8 μl and 19.7% for A. pruinosa, and 15 μl and 20% for A. greatheadii var. davyana) (Human and Nicolson, 2008).

Fig. 4. Relationship between plant size and inflorescences/plant (log₁₀ transformed) for (a) stem diameter and (b) height, from the five Aloe plicatilis populations in the south-western Cape, South Africa, where data on flowering were available (DKK, DKM, GB, GS and V1), combined.

| Windmeul 1 (W1)  | n   | Jason’s Hill (JH) | n   | Goudini Badsberg (GB) | n   | N | F  | p      |
|-----------------|-----|-------------------|-----|-----------------------|-----|---|----|--------|
| Nearest neighbour distance (m) | 2.59 ± 2.14 | 85 | 6.88 ± 7.91 | 29 | 2.33 ± 2.10 | 26 | 2, 137 | 100.25 | <0.001 |
| Pedicels bearing fruit/inflorescence (%) | 17 ± 11 | 15 | 13 ± 17 | 17 | 17 ± 16 | 13 | 2, 42 | 0.51 | 0.606 |
| Height of flowering individuals (m) | 1.37 ± 0.43 | 23 | 1.89 ± 0.51 | 14 | 1.33 ± 0.39 | 14 | 2, 48 | 7.45 | 0.002 |
| Number of inflorescences/individual = [A] | 6 ± 5 (1–22) | 23 | 8 ± 9 (1–32) | 14 | 4 ± 3 (1–13) | 14 | 2, 48 | 1.69 | 0.195 |
| Number of seeds/inflorescence = [B] | 41 ± 51 (6–146) | 15 | 16 ± 27 (4–84) | 17 | 50 ± 49 (9–146) | 13 | 2, 42 | 2.60 | 0.086 |
| Number of seeds/plant = [A] × [B] = [C] | 246 (41–902) | – | 128 (16–512) | – | 200 (50–650) | – | – | – | – |
| Seed production/population = [C] × (percentage of flowering plants in population) | 251,616 | – | 1325 | – | 27,930 | – | – | – | – |

Table 1 Differences in population size, nearest neighbour distance, natural fruit and seed set, flowering height, and total seed production in three populations of Aloe plicatilis in the south-western Cape, South Africa. Population size estimates were determined using a combination of the point-centred quarter (PCQ) and nearest neighbour methods. Fruit set is expressed as the percentage of pedicels/inflorescence that produced fruit and seed set as the mean number of seeds/fruit and number of seeds/inflorescence. Data are presented as X ± S.D., ranges are shown in brackets, and bold text indicates a significant difference.
Wilson et al., 2009). Human and Nicolson (2008) emphasise that even when floral characteristics are suggestive of specialist nectarivore pollination (e.g. as in A. greatheadii, var. davyana), honeybees can be the primary pollinators and their contribution to pollination in aloes in general should therefore not be discounted.

The accessibility of nectar to pollinators is important to consider when determining the pollinator guild of a particular species (Arena et al., 2013). In Aloe pluridens Haw. and Aloe lineata (Aiton) Haw. var. muirii (Marloth) Reynolds, for example, the anthers are included and adpressed at the mouth of the perianth, thus forcing bees to crawl over them to reach the nectar (Botes et al., 2009). Hence, these species are more likely to be pollinated by nectar-feeding bees than species with strongly exerted anthers such as Aloe africana Mill., Aloe ferox Mill. and Aloe speciosa Baker. Since the anthers and stigmas of A. plicatilis flowers are only slightly exerted (1–2 mm and 3–5 mm, respectively) (Reynolds, 1969) (Fig. 2f), they are therefore similar to the flowers of A. pluridens and A. lineata var. muirii, further supporting the results of this study that suggest that bees may be important pollinators for this species. Nonetheless, the significantly greater number of seeds/fruit in the ‘all visitors’ treatment possibly implies that despite the apparent importance of insects for the overall reproductive success of A. plicatilis, the bird contribution to pollination is significant at the level of the individual fruit. It is also possible that birds were able to extend their beaks through the cages of the ‘insects only’ treatment (as Stokes and Yeaton, 1995 observed in A. ferox) and hence may have elevated pollination success in these inflorescences. Furthermore, of the six South African aloe species, the floral morphology of A. plicatilis and Aloe tongaensis Van Jaarsv. (i.e. long, slightly curved flowers pollinated by specialist nectarivores (±50 mm) are consistent with pollination by long-billed nectarivores, since short-billed nectarivores would be unable to reach the nectar at the base of the flower. The Cape sugarbird (Promerops cafer) has a bill equally as long as that of the malachite sunbird (Geerts and Pauw, 2009), and has also been observed visiting A. plicatilis flowers (Nicolson and Roets, 2012). Other specialist nectarivores that occur within the distribution of A. plicatilis and are therefore possible visitors and/or pollinators include Cinnyris chalybeus (southern double-collared sunbird) and Anthobaphes violacea (orange-breasted sunbird), while Zosterops pullidus (Cape white-eye) is a possible opportunistic nectarivore visitor (Rebelo, 1987; Sinclair et al., 2011). However, these three species have comparatively short bills that are unlikely to reach the base of A. plicatilis flowers, and possibly rob nectar by piercing through the corolla tube as has been observed in other Cape plant species with similarly long corolla tubes (e.g. Chasmanthe floribunda (Salisb.) N.E.Br.) (Geerts and Pauw, 2009).

Mean nectar concentration at PB was double that at W1, suggesting some variability between populations. Overall nectar concentration was high (28%), and exceeded the upper limit of the range generally produced by flowers pollinated by specialist nectarivores (15–25% w/w) (Symes and Nicolson, 2008). Hence, in terms of the bird contribution to the pollination of A. plicatilis, the species’ floral morphology, its concentrated nectar, and observations of sunbirds and sugarbirds as floral visitors support the hypothesis that specialist long-billed nectarivores are more important than short-billed occasional nectarivores. However, further floral visitor observations and exclusion experiments at various A. plicatilis populations over several flowering seasons would help clarify its pollination system further, particularly patterns in the relative contributions of birds and insects to reproductive success.

In terms of the potential for self-compatibility in A. plicatilis, the total exclusion treatment yielded very low fruit and seed set (mean of 2% fruit set and 7 seeds/inflorescence), which suggests a small degree of autonomous self-pollination. However, low reproductive success at JH suggests an inability to self-pollinate, for if isolated flowering plants were self-compatible, greater fruit and seed set in these individuals would be expected. Self-incompatibility is widespread in the genus Aloe; though some recent studies suggest this may not be the case for certain species. Autonomous self-pollination has been observed in Aloe maculata All., Aloe krausii Baker, (Hargreaves et al., 2012), and Aloe pelegreae Schönland (Arena et al., 2013). However, seed set/fruit was very low in all three species, especially A. maculata and A. krausii (0.02 and 0.11 seeds/fruit, respectively). While self-compatibility

### Table 2

Summary of wind conditions at weather stations throughout the range of Aloe plicatilis in the south-western Cape, South Africa and potential seed dispersal distances for plants of three different heights. Wind conditions include annual predominant wind direction, mean monthly wind speed during the peak seed dispersal period (December), and the percentage of this period during which windless conditions prevailed. Potential seed dispersal distances were calculated using the formula in Stokes and Yeaton (1995). (Plant heights: 0.8 m = average height of A. plicatilis at the onset of reproduction, 1.23 m = mean height of reproductively mature individuals at populations DKK, DKM, GR, G5 and V1 (see Fig. 1) and 4.0 m = height of the tallest A. plicatilis individual encountered in this study).

| Nearest town (station name) | Altitude (m) | Predominant wind direction | Windless conditions (%) | Mean ± S.D. monthly wind speed (km/h) | Potential dispersal distance (m) |
|-----------------------------|--------------|---------------------------|------------------------|---------------------------------------|----------------------------------|
| Franschhoek (La Motte)      | 207          | NW and SW                 | 60.8                   | 4.5 ± 1.0                             | 0.8 m tall individuals            |
| Grabouw (Eikenhof)          | 365          | SE                        | 34.2                   | 7.8 ± 2.6                             | 1.23 m tall individuals           |
| Paarl (Mieweg)              | 102          | S                         | 9.2                    | 10.4 ± 0.9                            | 4.0 m tall individuals            |
| Rawsonville (Blaarfontein)  | 276          | SE                        | 40.2                   | –                                     | –                                |
| Stellenbosch (Nietvoorbij)  | 149          | SW                        | 21.0                   | 9.9 ± 1.6                             | –                                |
| Wolseley (LaPlaisant)       | 283          | E                         | 29.2                   | 10.0 ± 1.5                            | –                                |
| Worcester (Nuy)             | 225          | SE                        | 27.2                   | 9.1 ± 1.4                             | –                                |

### Table 3

Total percentage emergence of A. plicatilis seedlings after six months in three different germination media under ambient nursery conditions at the Karoo Desert National Botanical Gardens in Worcester, Western Cape, South Africa.

| Germination medium             | Emerged seedlings (%) |
|--------------------------------|-----------------------|
|                                | Fresh seeds (1-month-old) | 1-year-old seeds |
| Coarse river sand              | 72                     | 44                 |
| 50% fine river sand and 50% fine compost | 56 | 36                  |
| Soil from wild population      | 72                     | 52                 |
| Average percentage emergence   | 67                     | 44                 |
appears to be possible in A. plicatilis, detailed supplemental self- and cross-pollination experiments are necessary to confirm its breeding system.

4.2. Relationship between plant size and inflorescence production

Plant size is generally closely correlated with total flower production, with the largest plants in a population usually being the most floriferous (Ollerton and Lack, 1998). This relationship was evident in A. plicatilis, with larger plants (as measured by stem diameter and height) having more inflorescences than smaller ones. A similar trend has been demonstrated in studies on other long-lived succulent plants that are functionally similar to A. plicatilis: Pfab and Witkowski (1999) found a significant, positive relationship between canopy area and flower and fruit production in Euphorbia clevelandii R.A. Dyer, while McIntosh (2002) showed that the number of flowers produced by two Ferocactus species (Cactaceae) increased with plant size. In terms of other Aloe species, Hoffman (1988) recorded a significant positive linear relationship between plant height and raceme number, raceme length and fruit set in A. ferox, and Symes (2012) demonstrated that larger Aloe marlothii A. Berger individuals produced more seeds than smaller ones.

Klinkhamer et al. (1992) suggested that the relationship between plant size and reproductive output is curvilinear. Initial curvilinear relationships between plant size and inflorescence production determined for A. plicatilis produced a good fit, but the coefficient of determination was stronger using a linear regression with the dependent variable logarithmic transformed. Bazzaz et al. (2000), however, cautioned that the reproductive individuals in a population can vary enormously in size, owing to fine-scale environmental heterogeneity and competitive interactions, which leads to inequalities in the distribution of resources within a population. Hence, while the relationship between plant size and flower production in A. plicatilis is curvilinear, large plants may not always produce more flowers than smaller ones owing to differences in local environmental conditions (e.g. climate, shading by other species and nutrient availability).

4.3. Natural fruit and seed set

Natural fruit set at GB, JH and W1 was comparable to that measured for Aloe divaricata A. Berger, Aloe linearifolia A. Berger and A. marlothii (15, 19 and 12–18%, respectively) (Ratsirarson, 1995; Botes et al., 2009; Symes et al., 2009), but was less than half that of A. greatheadii var. dayana, A. pruinosa and A. peglerae (45–55, 41 and 40%, respectively) (Symes et al., 2009; Wilson et al., 2009; Arena et al., 2013). Number of seeds/fruit, was, however only comparable to that found for certain grass aloe species e.g. A. inopinata, A. minima and A. linearifolia (ca. 3.5, 10 and 13.5 seeds/fruit, respectively) (Hargreaves et al., 2008; Botes et al., 2009), and was 2–9 times lower than that of A. peglerae, A. greatheadii var. dayana, A. marlothii and A. pruinosa (all >34 seeds/fruit) (Symes et al., 2009; Wilson et al., 2009; Arena et al., 2013). Furthermore, estimated seed production/plant in A. plicatilis was far lower than that estimated for A. marlothii (95,148 for individuals <2.5 m and 167, 549 for individuals >2.5 m; Symes, 2012) and A. peglerae (3869; Arena et al., 2013). However, all the above-mentioned species are variable in terms of growth form, inflorescence size and shape and number of flowers/inflorescence, making comparisons between species difficult. Nonetheless, seed production in A. plicatilis, both at the level of individual fruit and whole plant, does appear to be markedly low relative to the size of flowering individuals.

JH exhibited the highest number of flowers/plant, but also the lowest fruit set/inflorescence and the lowest seed set/plant and per population. At GB and W1, almost all inflorescences on flowering individuals observed during population surveys produced at least one fruit, whereas a remarkably large proportion of inflorescences on most flowering individuals at JH died-back after flowering (i.e. functional in terms of producing pollen, but not fruiting). Post-survey analyses of fruiting plants at JH for which photographs were available (n = 8; 57% of all flowering plants in the population) revealed an average of 63% dead inflorescences/plant, and dead inflorescences comprised 77% of the total number of inflorescences of all eight plants combined. Furthermore, inflorescences that did produce fruit were often highly predated by birds (S.R. Cousins pers. obs.). Due to the uncertainty regarding the season in which the dead inflorescences were produced, only inflorescences that bore at least one fruit were sampled in order to determine fruit and seed set. Thus, while measures of reproductive output at JH were not significantly different from those of GB and W1, the results for JH probably overestimate the overall reproductive success at this population due to the unsampled dead inflorescences. Another indicator of reproductive failure at JH is that recruitment appears to be deficient, as surveys of population size structure (Cousins et al. submitted) revealed steep J-shaped stem diameter and height size class distributions indicating a preponderance of large adults and very little recent recruitment. Furthermore, plants at JH were, on average, 7 m apart, which differed significantly from the much shorter nearest neighbour distances of 2.6 m and 2.3 m measured for W1 and GB, respectively, and JH comprised only 31 plants, compared to ~399 at GB and ~3527 at W1 (Table 1).

Due to poor reproductive output, a lack of recent recruitment, small population size and large inter-plant distances at JH, it appears that the population is displaying the Allee effect. A. plicatilis individuals at JH may be too few and too far apart to attract sufficient pollinators, resulting in pollen and/or pollination limitation and consequent reproductive failure (Kunin, 1992; Ågren, 1996; Wilcock and Neiland, 2002). Furthermore, since aloes are generally self-incompatible, and the pollinator exclusion results suggest that this is likely the case for A. plicatilis, reproductive failure may be exacerbated as isolated individuals cannot self-pollinate for reproductive assurance (Knight et al., 2005). The A. plicatilis population at JH may have reached its Allee threshold (see Berec et al., 2007), and could undergo local extirpation if reproductive failure persists. However, repeat surveys of this population’s size structure and reproductive output may help to determine whether it displays poor annual reproductive success, and may assist in establishing how fruit and seed set relate to recruitment patterns and population structure.

4.4. Seed dispersal potential

Seed terminal velocity of 2.7 m/s for A. plicatilis was comparable to the 2.0 m/s calculated for Aloe candelabrum A. Berger seed by Stokes and Yeaton (1995). Potential dispersal distances were similar across the study area within plant height categories, and were approximately three times greater than plant height on average. Direct measurements of wind dispersal of the seeds of many plant species in the field show that most are dispersed very short distances, and usually fall near parent plants (Fenner and Thompson, 2006; Howe and Miriti, 2004; Corlett, 2009). Seed shadow patterns for all modes of dispersal are generally described by negative exponential functions (Willson, 1993), with the modal distance of wind-dispersed seeds being approximately equal to canopy height (Nathan et al., 2002). However, the estimated potential dispersal distances for A. candelabrum seeds calculated by Stokes and Yeaton (1995) were three-fold the height of seed release — a result that concurs with that calculated for A. plicatilis. This difference may possibly be due to aloe seeds generally possessing wings that aid wind dispersal (Jordan, 1999) and are therefore likely to travel further than seeds without any wind dispersal adaptations.

While short-distance seed dispersal influences the local dynamics of plant populations, long-distance dispersal is integral to their large-scale dynamics (Schurr et al., 2009). The “Cape Doctor”, or south-easterly wind, blows over the south-western Cape during summer, when seed dispersal in A. plicatilis occurs, and is known to be persistent
and often very gusty, with gusts of up to 35 m/s (128 km/h) recorded in Cape Town (Kruger et al., 2010). Since the geographical distribution of A. plicatilis is very patchy, with many populations separated by large distances, it is possible that these isolated populations arose due to long-distance dispersal events caused by strong gusts of wind. Arguably, isolated A. plicatilis populations that occur on continuous mountain ranges such as the Du Toit’s Kloof and Franschhoek mountains may once have been connected and subsequently become fragmented due to changing climatic conditions over time (see citations in McClauchlan and Clark, 2004).

However, the occurrence of populations on mountains completely disconnected from other more continuous mountain ranges e.g. PB on the Paardeberg and W1 and W2 on Paarl Mountain raises questions regarding the possibility of long-distance dispersal. Schurr et al. (2009) noted that for a plant species to persist in a fragmented landscape (such as a mosaic of mountain and lowland habitats), local extinction from occupied habitat fragments must be balanced by the colonisation of unoccupied fragments, which requires long-distance dispersal to those fragments. Horn et al. (2001) emphasized that many of the aerodynamic mechanisms that facilitate long-distance dispersal in light, fluffy or plumed seeds may also apply to heavier winged seeds (such as aloe seeds) at somewhat different scales. Furthermore, turbulence and variations in wind velocities may more frequently and more extensively promote extreme dispersal than retard it despite reducing the modal dispersal distance on average (Horn et al., 2001). However, in order for seeds to be dispersed long distances they must be captured in convection cells of the scale of hundreds of metres to kilometres (Horn et al., 2001).

Since A. plicatilis occurs exclusively on well-drained, rocky mountain slopes that act as fire refugia, it is unlikely that it historically occurred in the less-rocky lowland fynbos between the Paardeberg, Paarl Mountain and the Du Toit’s Kloof/Franschhoek mountains. Furthermore, both the Paardeberg and Paarl Mountain occur north-west of several other large A. plicatilis populations (e.g. LM, TWK and ZH), from which seed may have been transported by strong south-easterly winds. Testing this hypothesis may prove challenging, as long-distance dispersal events are very rare and difficult to track (Horn et al., 2001; Nathan et al., 2002), although analyses of population genetic variation (e.g. He et al., 2004) may provide a feasible approach.

4.5. Seed banks and seedling recruitment

The total absence of seedling emergence from the soil samples from most populations of A. plicatilis suggests that the species forms only transient seed banks that last for < 1 year after dispersal. There was also little or no evidence of recruitment in situ or in the soil samples collected below plants that had many spent in fynbos. Such observations were made for shaded versus non-shaded A. plicatilis seeds sown outdoors in seed beds either under an organic mulch or under shade netting exhibited greater rates of seedlings emergence, and seedlings were considerably greener, more turgid and their leaves were approximately four times longer than the those of seedlings grown in full sun.

Similar observations were made for shaded versus non-shaded A. plicatilis seedlings growing in an environmental control chamber and greenhouse (S.R. Cousins, pers. obs.), and for A. plegerea under the same conditions (G. Arena, pers. comm.). Rodríguez-García et al. (2007) found that the new leaves of Aloe vera Mill. plants are sensitive to water stress and Bairu et al. (2009) showed that regular watering of Aloe ferox Mill. seedlings enhanced most of the growth parameters studied. A. plicatilis seedlings in cultivation also respond well to regular watering and may show considerably reduced turgidity after ± 2 weeks without watering (S.R. Cousins, pers. obs.). These findings suggest that despite having succulent leaves that act as a buffer against desiccation, in order for A. plicatilis seedlings to establish and persist in wild populations they require substantial, consistent rainfall over winter and probably into spring and early summer in order to accumulate sufficient water reserves to survive the subsequent summer drought.

4.6. Germination trials

4.6.1. Controlled and ambient laboratory conditions

Despite the 3-month-old A. plicatilis seeds germinating approximately three times faster than both the 18- and 24-month-old seeds, total germination in the former was three-fold less than the latter two (Fig. 5). The tetrazolium tests showed that 100% of the remaining ungerminated 3-month-old seeds that were not empty were viable. These findings suggest that A. plicatilis seeds undergo after-ripening —
a progressive loss of primary dormancy in air-dry seeds, which is a function of environmental variables and time (Murdoch and Ellis, 2000). Bairu et al. (2009) suggested the need for an after-ripening period in A. ferox seeds, since total germination at various temperatures, light intensities and concentrations of plant growth regulators was <80% after ±1 month, although 95% of ungerminated seeds were viable. Staggered germination was also observed in A. greatheadii var. davayana and A. marlothii seeds, some of the former still germinating in the second season after sowing (Smith and Correia, 1992; Symes, 2012). Ellof and Liede (1987) reported increased percentage germination in A. dichotoma, Acacia karroo and A. marlothii seeds, some of the former still germinating in the second season after sowing (Smith and Correia, 1992; Symes, 2012). Ellof and Liede (1987) reported increased percentage germination in A. dichotoma, A. speciosa, Aloe thraskii Baker and Aloe vyreheidensis Groenew. seeds stored for ±16 months under ambient conditions compared to those stored for only four months. However percentage germination in other Aloe species studied either decreased or remained fairly consistent over time.

Although the vigour of the 18- and 24-month-old A. plicatilis seeds in this study decreased slightly (indicated by slower germination rates), their viability was maintained. This result is, however, in stark contrast to the extremely poor germination of 0.5% Ellof and Liede (1987) obtained for A. plicatilis seed stored for 16.5 months under ambient laboratory conditions. However, the initial viability of that particular seed lot was unknown and the reasons for germination failure were therefore unclear. While the potential longevity of seeds stored in an air-dried state in a laboratory may provide clues about their longevity in the field, laboratory-stored seeds are exposed to much smaller variations in temperature, moisture and solar radiation compared to those in habitat (which are also vulnerable to predation). Seeds dispersed in habitat therefore probably survive for much shorter periods. Differences in viability between laboratory-stored seeds and those in the wild have been shown for marula (Sclerocarya birrea (A. Rich.) Hochst. subsp. caffra (Sond.) Kokwaro), which remain viable for many years in a laboratory, but form small, short-term persistent seed banks in the wild (Helm et al., 2011). In addition, Mbalo and Witkowski (1997) demonstrated the effect of simulated high soil surface temperatures on seeds of Acacia karroo Hayne, Acacia tortilis (Forssk.) Hayne subsp. heteracantha (Burch.) Brenan and Chromolaena odorata (L.) K. & R., which all declined in seed viability with increasing duration of exposure at 70 °C. While the viability of A. plicatilis seeds in the wild is most likely restricted to <1 year, seed storage and germination experiments in situ would help validate this deduction and thus contribute to our understanding of regeneration and establishment not only in A. plicatilis, but also aloes in general.

The percentage of one-year-old seeds that germinated and emerged under nursery conditions was fairly low (44%) compared with fresh seeds (67%), which suggests that some of the one-year-old seeds may have lost viability during storage or during the germination trials. The two germination media that appeared to favour the germination and emergence of A. plicatilis seeds were air-dried sand and soil from the wild population, as they both yielded higher percentage emergence than the mixture of fine river sand and compost. The low percentage emergence of seeds under nursery conditions may have been due to fluctuating temperatures and relative humidity outside in the Karoo Desert National Botanical Garden, compared with seeds germinated under more stable conditions in the environmental control chamber and laboratory. For optimal germination and emergence it is therefore recommended that A. plicatilis seeds less than one year old be sown under controlled conditions in a growth chamber or greenhouse.

5. Conclusion

A. plicatilis appears to be pollinated primarily by insects; however, bird visitation significantly increased seed set/fruit, suggesting co-pollination with insects. Malachite sunbirds appear to be the most common avian visitors to A. plicatilis flowers — an observation that is consistent with the species’ floral morphology and concentrated nectar which suggest pollination by specialist avian nectarivores. Natural seed set at three populations varied by orders of magnitude, and reproductive success was poorest at the smallest and least dense population. The reproductive failure evident at this population suggests a possible Allee effect, but this requires further investigation. Potential seed dispersal distances in A. plicatilis were estimated to be approximately three times canopy height, consistent with a negative exponential seed shadow. However, the species’ occurrence on isolated mountains that are disconnected from more continuous mountain ranges where the species also occurs suggests that long-distance dispersal by strong, gusty summer winds is possible. Seed banks in A. plicatilis populations appear to be transient, lasting for <1 year, and recruitment is likely erratic and ‘disturbance free’. A. plicatilis seeds stored under ambient laboratory conditions for 18 and 24 months maintained high viability with total germination at ≥80%, while that of 3-month-old seed was three times less, suggesting the necessity for after-ripening. Emergence of A. plicatilis seeds under nursery conditions was not as successful as germination under controlled conditions in a growth chamber and laboratory. Further pollinator exclusion experiments and floral visitor observations will help verify the species’ pollinator guild, and detailed supplemental self- and cross-pollination experiments are required to confirm its breeding system. Very little is known about the germination ecology of A. plicatilis (and most other aloes) in habitat. Further studies on germination requirements and recruitment patterns in the genus in situ would be especially beneficial for the conservation of rare and threatened Aloe species.

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