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Improving success rates of remote conservation translocations by mitigating harsh *in-situ* environmental conditions: A case study on a Critically Endangered succulent

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ABSTRACT

Conservation translocations of threatened plants are an important measure used to curb extinctions. Some translocation attempts have had poor success rates, particularly in remote locations where after-care is difficult, and this type of intervention often lacks empirical data to inform protocols for subsequent attempts. To address this issue, we undertook a conservation translocation on a Critically Endangered succulent (*Aloe peglerae*), with the aim of identifying factors that promote survival rates of transplanted seedlings in remote areas of suitable habitat. Protection from the sun, through shading by grass tussocks, improved survival rates of transplanted seedlings significantly during all three years within the study period. Survival rates were also higher for larger seedlings, but the latter effect was only observed during the first-year post-transplantation. Seedling growth differed between years (being highest in the wettest year), and was improved by the applications of potassium silicate (K_2SiO_3) during cultivation, a biostimulant that increases drought tolerance in plants. All the observed positive effects on seedling growth were, however, only significant during the first-year post-transplantation. Fire exposure negatively affected seedling growth, but also only during the first-year. Based on these results, a suggested protocol for guiding the planning and implementation of conservation translocations of threatened succulent plant species is provided. This study illustrates the value of generating empirical data prior to undertaking larger scale conservation translocations to maximise resource use and increase success rates. The use of a biostimulant is a novel approach to the field of conservation translocations, and could have wide-ranging applications.

1. Introduction

Anthropogenic activities are having increasingly negative effects on global biodiversity (Johnson et al., 2017), including dramatic global reductions of population sizes of numerous plant species (Braje & Erlandson, 2013), and escalating numbers of extinctions (De Vos et al., 2015). Plant species make up some of the most threatened organisms on Earth (Gilbert, 2010; Willis, 2017), facing multiple anthropogenic threats including habitat transformation (Cagnolo et al., 2006; Hahs et al., 2009; Rouget et al., 2003), anthropogenic climate change (Brusca et al., 2013; Dullinger et al., 2012; Parmesan & Hanley, 2015), and widespread poaching for both medicinal (Hannweg et al., 2016; Voeks & Leony, 2004) and horticultural reasons (Bamigboye et al., 2016; Remucal, 2015). Anthropogenic climate change has multifaceted negative effects on many plant species, with the effects often occurring over long periods of time (Parmesan & Hanley, 2015). In contrast, poaching can have more rapidly tangible effects, with many plant species having been poached to extinction in the wild (IUCN, 2023; Pfab et al., 2016; Santos-Díaz et al., 2010), often within just a few decades or less. A high number of plants are under threat from poaching because of their rarity and associated monetary value to plant collectors and traders (Pfab et al., 2016; Smith et al., 2023). Additionally, poaching can also have knock-on effects on animal species with which the targeted plants interact (Janse van Rensburg et al., 2023; Schleuning et al., 2015).

The practice of conservation translocations (IUCN/SSC, 2013) is one initiative that has been used to aid the conservation of plant species that have been subject to poaching-induced population declines and extinctions (Fath, 2018; Maunder, 1992). Conservation translocations consist of three primary practices, namely reinforcement, reintroduction, and assisted colonisation (IUCN/SSC, 2013). With relevance to this study,

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reinforcement is defined as the intentional movement and establishment of organisms into existing populations (see e.g. Baruah et al., 2019), and deals with the bolstering of extant populations of threatened species.

While conservation translocations may potentially be a key component in the conservation of threatened plant species, there is a general paucity of documented studies, which challenges the advance of the practice. Indeed, the varied success rates (Bellis et al., 2024) of conservation translocations have been partly been attributed to a lack of reliable protocols that are based on empirical data (Julien et al., 2022). Additionally, to determine if a translocation was truly successful or not, extended monitoring periods are required, for at least the time required for flowering, seed production, and subsequent recruitment to be detected (Monks et al., 2012). To date, few studies have monitored the outcomes of translocations over such extended timeframes (Julien et al., 2023), and short-term results can still be valuable (Commander et al., 2018; Doyle et al., 2023; Monks et al., 2012). In addition to the challenge of sufficient monitoring times, the lack of documentation of translocation attempts is likely due to numerous other practical challenges associated with the practise (Godefroid et al., 2011), such as the often remote nature of the natural habitats where translocations need to be undertaken (Coopoosamy, 2011), which can place financial and logistical constraints on the number of propagules that can be translocated, reducing the feasibility of post-planting care (D'Agostino et al., 2024). The climatically harsh nature of many natural habitats where threatened plants occur is an additional challenge that can negatively affect short- and long-term success rates (see e.g. López-Jurado et al., 2019). Thus, improved assessment and reporting of translocation protocols can potentially maximise resource use in conservation translocations.

Although poaching is a global conservation concern, plant poaching is often more prevalent in areas with high levels of plant diversity and endemism. Southern Africa is a prime example of this trend, where over 60 % of the 21,100 vascular plant species of the region are endemic (Klopper, 2010; Steenkamp & Smith, 2006; van Wyk & Smith, 2001), and where a large number of these are classified as of conservation concern (Near Threatened, Vulnerable, Endangered or Critically Endangered) because of poaching activities (Pfab et al., 2016). Additionally, several plant species in this region have been declared extinct in the wild, many cases of which can be attributed to poaching (Pfab et al., 2016). The unique and highly diverse succulent flora of southern Africa (Klopper, 2010; van Wyk & Smith, 2001) have been particularly adversely affected by poaching (Pfab et al., 2016; Smith et al., 2023), with species in the Aizoaceae, Asphodelaceae, Crassulaceae, and Euphorbiaceae families being highly targeted by poachers (Smith et al., 2023). This trend has increased in recent years as a result of poor economic conditions and high unemployment levels (Smith et al., 2023). The succulent genus *Aloe* L. (Asphodelaceae), is a group of plants that is often targeted by poachers (Arena et al., 2017; Pfab & Scholes, 2004). The genus comprises around 575 taxa (Grace & Klopper, 2014), and is primarily distributed in sub-Saharan Africa, the Arabian Peninsula, and Madagascar (Smith & van Wyk, 1991). The threatened nature of many *Aloe* taxa, and their associated horticultural demand, has resulted in the inclusion of all but one species (*Aloe vera* L. Burm.f.) on the CITES Appendixes A or B (CITES, 2019), which restricts international trade to curb poaching.

Besides the complex ethical incentives for conserving threatened plants (Baard & Ahteensuu, 2019; van Dyke & Lamb, 2020), there are several ecological reasons to do so too. *Aloe*, for example, are integral parts of many African ecosystems, as they are important food sources for a variety of vertebrates (De Swardt, 1991; Kuiper et al., 2015), and invertebrates (Botes et al., 2009; Human & Nicolson, 2008), primarily through the production of large amounts of nectar (Nicolson & Nepi, 2005). The majority of *Aloe* species flower in winter (van Wyk & Smith, 2014), when nectar and pollen is of particular importance as few other plant species are in flower (Human & Nicolson, 2008; Symes et al., 2008). The importance of *Aloe* in many ecosystems (Cousins & Witkowski, 2012; Midgley et al., 1997), and their threatened nature makes this charismatic genus (Grace et al., 2013) an ideal candidate for various conservation initiatives.

Despite the large number of threatened *Aloe* taxa, there have been no documented attempts to undertake conservation translocations on the genus. There have, however, been a limited number of studies using these practices on other succulent plant taxa, some of which showed promising results (Birnbaum et al., 2011; Caitano et al., 2022; Cypher et al., 2014; Earlé et al., 2017; García-Rubio & Malda-Barrera, 2010; Harris et al., 2014; Reemts et al., 2014; see e.g. Rubluo et al., 1993; Stiling et al., 2000), although few of these continued monitoring for longer than two years (Harris et al., 2014; Jasper et al., 2005; Reemts et al., 2014; van Jaarsveld, 1999). Of these, only two resulted in recruitment during the study period (Harris et al., 2014; Jasper et al., 2005).

To address the lack of knowledge on threatened succulent conservation translocations, we undertook an experimental reinforcement on the Critically Endangered *Aloe peglerae* Schönland by translocating cultivated seedlings into an extant population of the species. *Aloe peglerae* is a suitable model species that will inform best practices for conservation translocations on threatened *Aloe* and other succulent species' conservation, and meets the criteria to be considered a conservation flagship species owing to its conservation status, South African endemic status, rarity, ecological function value, public interest, and its socio-economic and cultural value (Qian et al., 2020).

To determine the best practices for conservation translocations on this, and potentially other threatened succulent species, the objectives of this study were to determine whether 1) microsite selection, 2) seedling size, and 3) biostimulant application (to increase drought tolerance) affect the survival and growth rates of translocated seedlings. We hypothesize that shaded microsites, larger seedlings, and biostimulant application will be conducive to higher survival and growth rates of translocated seedlings. These findings can then inform larger-scale translocation efforts for *A. peglerae*, and act as a starting point for experimental translocation studies on other succulent species.

2. Methods

2.1. *Study site and species*

The study was conducted in the Magaliesberg mountain range in the North-West province of South Africa, which is c. 50 km in length and reaches a maximum altitude of 1 850 m a.s.l. The Magaliesberg lies in an ecotone zone (Sarthou et al., 2010), (Fig. 1), between the Savanna and Grassland biomes (Mucina & Rutherford, 2006). This habitat (Fig. 2a) is characterized by steep and rocky east–west orientated quartzite ridges with acidic, nutrient poor and shallow (usually of a depth no greater than 0.15 m) lithosols (Pfab & Scholes, 2004). The shallow and rocky soils, coupled with the steep gradients in the habitat, means that even in the event of good rainfall, rapid runoff (Arena et al., 2015) may result in regularly dry soils. The climate of the Magaliesberg is characterized by summer rainfall, occurring between October and March, with an average of 791 mm per annum (Pfab & Scholes, 2004). Microclimatic differences occur across the mountain range, with the south-facing slopes being more shaded, cooler, and more mesic than north-facing slopes (Carruthers, 2000). Frost frequently occurs in winter but is usually restricted to the southern slopes and lower lying areas (Mucina & Rutherford, 2006). The average maximum temperature in the hottest month and average minimum temperature in the coldest month is 24° C and 10° C respectively (South African Weather Service, 2019). The Magaliesberg is a declared World Biosphere Reserve (Fig. 1b) (UNESCO, 2015).

Aloe peglerae Schönland (Fig. 2b) is a charismatic (Payne et al., 2017) succulent and one of the most threatened species in its genus. It is a stemless aloe that forms a single rosette of leaves with a margin of red spines (van Wyk & Smith, 2014). It is a slow growing and long-lived

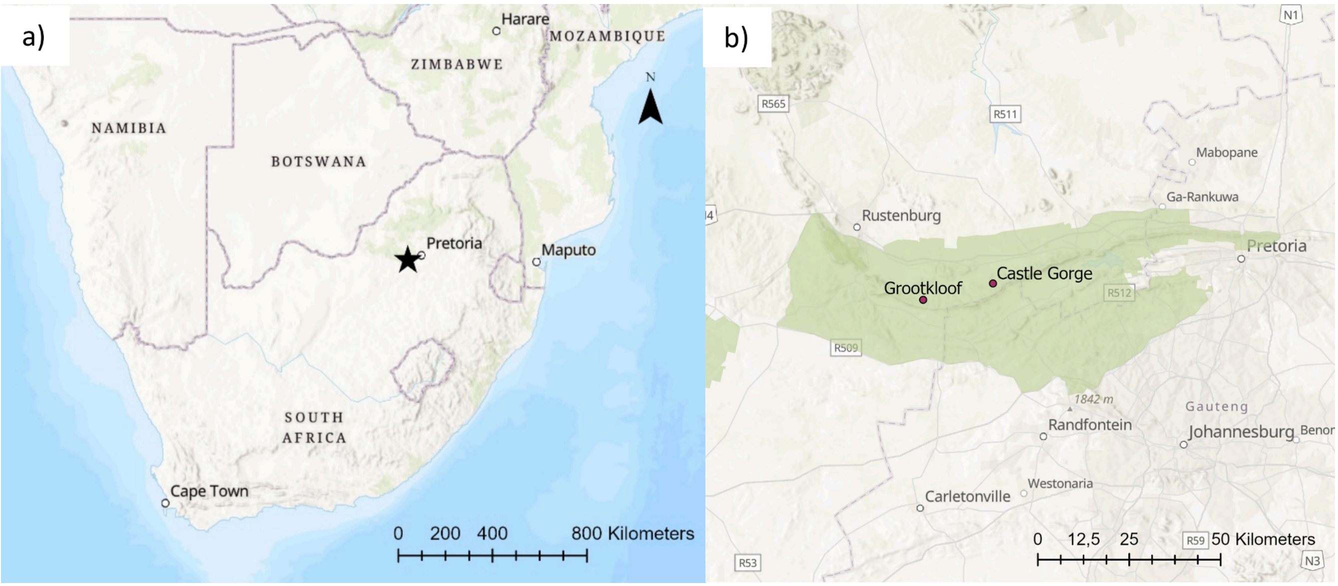

Fig. 1. A) the general location of the magaliesberg in southern africa, with theMagaliesberg Biosphere Reserve indicated by a star, and b) the extent of Magaliesberg Biosphere Reserve indicated in green with the location of the two study sites indicated (Grootkloof and Castle Gorge). The entire distribution of *A. peglerae* falls within the boundaries of the Magaliesberg Biosphere Reserve. Map sources: a) Esri South Africa, Esri, TomTom, Garmin, FAO, NOAA, USGS, Esri, USGS; b) Esri South Africa, Esri, TomTom, Garmin, FAO, NOAA, USGS, Esri, CGIAR.

species (~60 years) (Pfab & Scholes, 2004), with low levels of recruitment (Scholes, 1988), and a generation time of around 30–40 years (Pfab & Scholes, 2004). The species produces a dense cylindrical raceme inflorescence between July and August (Glen & Hardy, 2000), with flowers changing colour from red to green-yellow as they mature (van Wyk & Smith, 2014). This species is pollinated by birds, including the Cape rock-thrush (*Monticola rupestris*) and dark-capped bulbul (*Pycnonotus tricolor*), and also small mammals including the Namaqua rock mouse (*Micaelamys namaquensis*) and the eastern rock sengi (*Elephantulus myurus*) (Payne et al., 2016). An individual plant may produce thousands of seeds following pollination (Payne et al., 2016; Scholes, 1988). *Aloe peglerae* is often exposed to regular fires that occur in its habitat (van Wyk & Smith, 2014).

Although *A. peglerae* is known to have had a historically restricted distribution in the Magaliesberg and Witwartersberg where it is restricted to the steep upper northern grassy slopes (Payne et al., 2016; van Wyk & Smith, 2014), poaching for horticultural reasons has put this already uncommon species in danger of extinction in the wild (Pfab & Scholes, 2004). Populations more easily accessible to the public have been highly reduced through poaching of both plants and seed (Pfab & Scholes, 2004). The loss of even one plant to poaching per average population per year has been found to be unsustainable (Pfab & Scholes, 2004). In a study investigating the population trends of *A. peglerae* in nine locations, a 43 % decline was detected during the period of 1999 and 2010 (Phama et al., 2014), with an area of occupancy (AOO) of just 3415 km^2 (Pfab et al., 2016). In 2004, there were an estimated 70 000 mature individuals remaining in habitat (Pfab & Scholes, 2004). By extrapolating the observed rate of decline (Phama et al., 2014), it is likely that both the current AOO and number of mature individuals has decreased considerably by the time of this publication.

The species was last assessed for the IUCN Red List of Threatened Species in 2003, and categorized as Endangered (IUCN, 2023). More recently, however, it has been categorized as Critically Endangered on the Red List of South African Plants (the only country in which this species occurs) owing to the predicted population decline of over 80 % of within three generations (IUCN Standards and Petitions Committee, 2024; Pfab et al., 2016). Protecting the remaining plants *in-situ* would be the most cost-effective conservation measure, but the remote nature of their habitat, the incidental and opportunistic poaching trends, together with the lack of local conservation resources, means that such efforts are difficult to enforce (Pfab et al., 2016). Thus, to address the species' threatened status, alternative conservation measures are required, in addition to anti-poaching efforts (Pfab & Scholes, 2004).

2.2. *Propagation*

Aloe peglerae was propagated from habitat-collected seed that were collected annually for the duration of the study (North-West Province Permit: NW 16567) from two sites near the town of Mooinooi, North West province, South Africa (Grootkloof and Castle Gorge; these were also the two sites for transplantation, with seedlings only transplanted back to the sites from which their seed had been originally collected; Fig. 1b). Both sites had many mature individuals present, although no formal census has been conducted. Seeds were germinated and cultivated on a heated propagation bed in a polycarbonate tunnel at the University of Pretoria, South Africa. A sandy loam soil mixture was used to simulate substrate conditions found in *A. peglerae* habitat (Doyle et al., 2021). Seedlings were grown for one year in trays that house 90 cm^3 tubes (see e.g. Turner et al., 2021) lined with biodegradable gusset bags (see e.g. Nam et al., 2016; Shaari et al., 2020) (Fig. 2d and f). The tubes were cut longitudinally and resealed with duct tape to enable easier removal of the seedlings in an effort to reduce root disturbance during transplantation into the study sites. The gusset bags were used to keep the soil in place around the seedling roots during transplantation, thus further minimising transplantation shock (Silcock et al., 2019). Various other steps were taken to further reduce transplantation shock, for example, by maintaining the orientation of the seedlings throughout propagation and transplantation, so that the seedlings faced the same cardinal directions for the duration of the study. This step ensures that limited energy would be expended by the seedlings through alteration of their positive heliotropism (Darwin, 2009).

To further mitigate transplantation shock, an important factor to consider is the preconditioning of the seedlings prior to translocation to acclimate them to the harsh conditions of their natural habitats (Commander et al., 2018; Ortiz-Martínez et al., 2021; Vilagrosa et al., 2003). Nursery conditions are more favourable than *in-situ* conditions, particularly in terms of water availability and solar radiation (Valliere et al., 2019; Vilagrosa et al., 2003). Seedlings can be preconditioned to

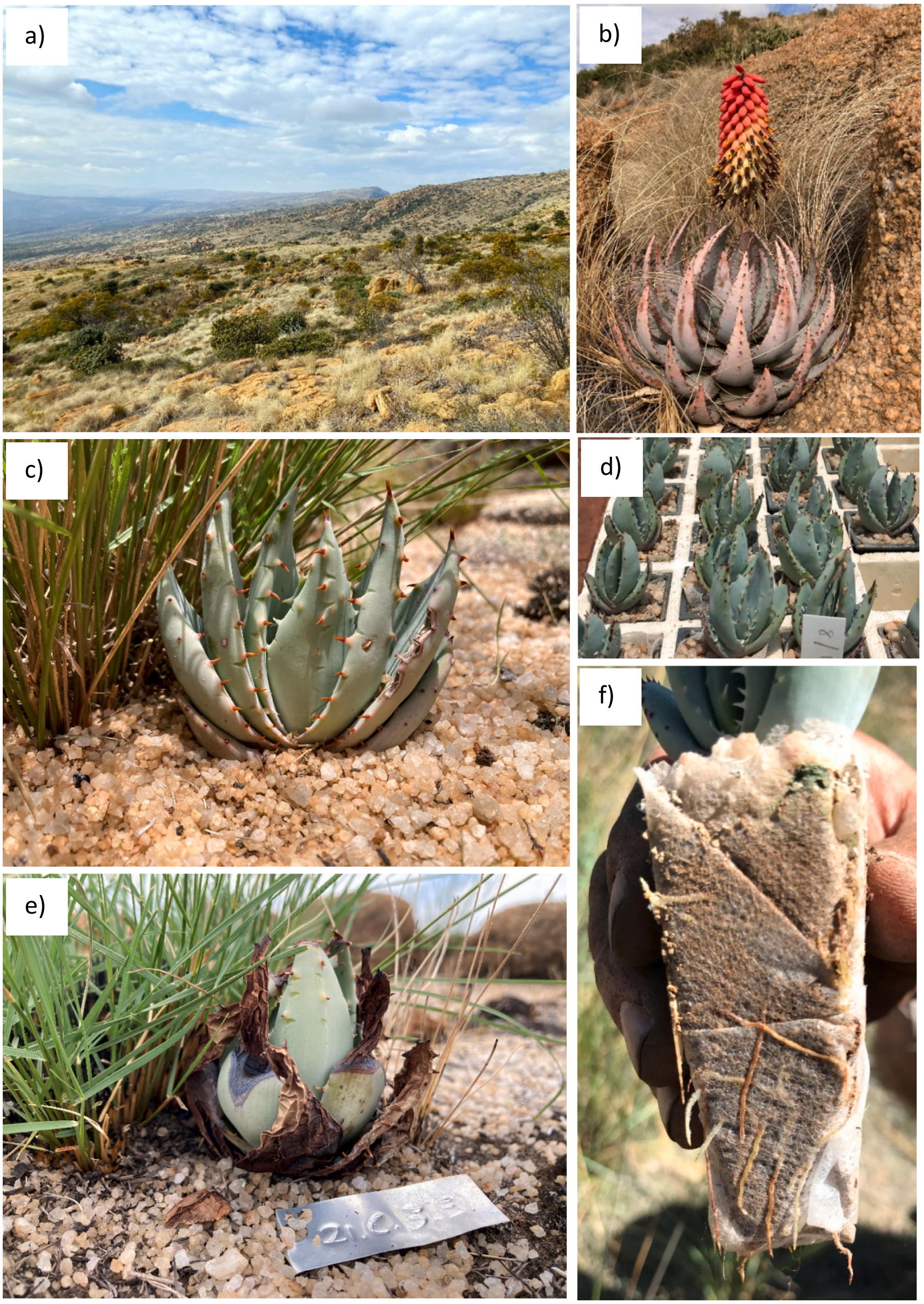

Fig. 2. A) typical*A. peglerae* habitat on the upper northern slopes of the Magaliesberg, b) an adult individual, c) a healthy seedling 1.5 years after transplantation, d) seedlings prior to transplantation, e) a seedling burnt in a fire, and f) a seedling with roots growing through a biodegradable gusset bag.

be more drought tolerant by gradually reducing water availability whilst simultaneously increasing radiance exposure, which may improve translocation success rates (van den Driessche, 1991; Vilagrosa et al., 2003). To precondition the *A. peglerae* seedlings to the harsher *in-situ* conditions prior to transplantation, the seedlings were watered three times a week (~300 ml/seedling) for the first nine months, after which the watering rate was reduced to once a week (~100 ml/seedling) for the final three months of propagation. Also, during the last three months, the seedlings were moved out of the polycarbonate tunnel to an outdoor netted (20 % shade) area where they were exposed to a less humid climate and higher solar radiance, but sheltered from rainwater by a clear polycarbonate roof.

To test a novel treatment aimed at addressing the expected negative effects of drought stress post-transplantation, half of the seedlings were treated with a biostimulant (Potassium Silicate, K_2SiO_3; hereafter abbreviated to PS) solution at a concentration of 1 ml PS/L H_2O once weekly for the last three months (September-November = 13 applications) of propagation. The PS was applied to the seedling as a foliar spray until all the leaves were covered by the solution and until the soil in the gusset bags were drenched (~100 ml PS solution/seedling). The application of PS in the form of a foliar spray and drench has been used successfully to increase drought-tolerance of plants, particularly in an agricultural context (Abu El-Azm & Youssef, 2015; Eneji et al., 2008). The mechanism of increased drought tolerance through PS application is linked to increased production of osmotic regulators and anti-oxidant activity (Feghhenabi et al., 2022; Oraee et al., 2023), as well as increasing relative water content (Eyni-Nargeseh et al., 2022). PS has also been shown to have various other benefits to plants, ranging from increased stomatal resistance, increased chlorophyll content (Eyni-Nargeseh et al., 2022), disease (El-Sheery, 2017) and pest resistance (Jeer et al., 2021), enhanced nutrient uptake (Ibrahim et al., 2020), and promotion of the development of physiological and anatomical traits associated with increased growth (Azab et al., 2022; Feghhenabi et al., 2022). The seedlings not treated with PS received a foliar spray of water in the manner described above (~100 ml H_2O) on the same days as PS was applied to ensure that all seedlings received the same amount of moisture. The PS application and additional water for untreated seedlings was integrated into the watering schedule already outlined.

Aloes are susceptible to attack from a variety of pests and diseases which are widespread in cultivation. These include various bacteria (De Laat et al., 1994; Mandal & Maiti, 2005), fungi (Ghosh et al., 2018; Kawuri et al., 2018; Roux et al., 2009), viruses (Reynolds, 2004), and arthropods (Kelly & Olsen, 2008; Light, 2012; Prinsloo & Uys, 2014). To ensure that the seedlings were free of pathogens and pests prior to being transplanted (Commander et al., 2018), they were treated monthly during propagation with a foliar spray and drench of 5 % Hydrogen peroxide (H_2O_2)–H_2O solution (~100 ml solution/seedling) to kill epiphytic and soilborne fungi, bacteria, and arthropods (such as *Aceria aloinis* Keifer), and a systemic fungicide (benzimidazole) suspension drench (1 g/L H2O; ~100 ml solution/seedling) to kill endophytic fungi. The pest- and disease-control applications were also integrated into the outlined watering schedule. As an additional precaution, pesticide granules (chloro-nicotinyl; 7,5 g/kg) were applied at a rate of ~1 g/seedling every three months between January and October (four applications) to kill invertebrate pests. Although chloro-nicotinyl is toxic to various non-detrimental invertebrates, its application was deemed necessary to ensure no pests including white scale insect and the aloe snout weevil were introduced into the study sites. This decision was further supported by the half-life of chloro-nicotinyl (Sarkar et al., 2001) being sufficiently short that the compound would have decomposed shortly after transplantation.

2.3. Translocation and monitoring

Translocations were undertaken at an altitude of ~1500 m a.s.l. on two privately owned properties 18 km apart, both which are within the Magaliesberg and Magaliesberg Biosphere Reserve (Fig. 1). Transplantation was undertaken in early December each year (2020–2022) to coincide with the start of the rainy season (Commander et al., 2018; Doyle et al., 2023). The dimensions (height, width, and diameter) of all the seedlings were recorded on the day prior to transplantation. Seedlings were planted at the site where their seed was originally collected to avoid genetic contamination (representing a population reinforcement). Seedlings were planted in predetermined clusters (i.e. locations within each site) which were chosen based on their biotic and abiotic characteristics indicating suitable microsites (Arena et al., 2015): soil of depth of 10–15 cm, high rock cover, low vegetation cover, the presence of grass tufts, the occurrence of bare (i.e. unvegetated) soil, as well as the presence of mature *A. peglerae* plants. In a study investigating the soil-stabilising potential of plant species in disturbed sites, the condition of *Aloe davyana* Schönl. var. *davyana* (Schönl.) Glen & Hardy seedlings germinated *in-situ* was better when they were in the shade to the east of a grass tuft (Smith & Correia, 1992). Thus, one of the treatments applied in this study was planting half of the seedlings in a shaded microsite on the eastern side of a grass tuft to protect them from the harshest afternoon sun.

Within each cluster, seedlings were planted representing four treatments: full sun and untreated with PS, full sun and treated with PS, shaded and untreated with PS, and shaded and treated with PS. Two replicates of the four treatments were planted in each cluster, with each cluster comprised of eight seedlings. Four clusters were planted in each of the two study sites in 2020, totalling 64 seedlings (Table 1). The above methods were repeated in 2021 and 2022 by supplementing the existing clusters with the same number of additional seedlings (representing all four treatments) (n = 192 seedlings across eight clusters).

When planting the seedlings, a trowel was used to excavate a ~90 cm^3 hole, with dimensions approximate to the seedling tubes. The seedlings were carefully removed from their tubes with the gusset bags by cutting the tape and folding open the tube, with caution taken to not disturb their roots (Fig. 2f). The seedlings were planted in the hole, and the surrounding soil was gently compacted to ensure good soil-root contact (Commander et al., 2018). Each seedling received 20 ml of water thereafter, which was the only water provided for the remainder of the experiment.

Each consecutive December after being planted, the number of surviving seedlings were recorded together with their dimensions (length, width, and height). With these measurements, the volume of each seedling was estimated at different periods using the formula for an ellipsoid (Volume $= \frac{4}{3}\ x\ \pi\ x$ length x width x height), as this shape is the closest fit to the shape of the seedlings. The volume of translocated plants is often used together with survival rates as a proxy for translocation success (Monks et al., 2012), particularly when assessing establishment success.

Climatic data from the nearest weather station (~9.5 km from both study sites) were sourced from the South African Weather Service for the period of December 2020 to December 2023. Natural fires occurred in both sites during the winter months (June-August) between 2020 and 2023. These fires were unplanned, and thus no assessments could be made to determine the intensity of the fires. Additionally, the fires were patchy in nature, with some seedlings being exposed to fire and others not. Following these unplanned fires, the effects of fire were included in analyses of survival and growth of the transplanted aloes. Many aloes lost the shading provided by grasses following a fire, but as the burnt grasses resprouted and grew to sizes large enough to again provide shade within three months, the effect of a short period without shade was excluded from the analyses.

2.4. Data analysis

The effect of the following predictor variables on seedling survival one, two, and three years after being transplanted were tested: light exposure (shaded vs open microsite), fire exposure (burnt or not), PS treatment (PS applied or not), year planted (2020, 2021 or 2022), and volume when transplanted (cm^3). The variance inflation factor (VIF) was calculated to identify any predictors showing strong multicollinearity. None of the predictor variables were strongly collinear (VIF <3; Tables A4 and A6).

The analyses of survival were conducted using Generalized Linear Mixed Models (GLMM) with a binomial distribution. These models used cumulative survival data, as opposed to survival data for specific years

Table 1
The number of *Aloe peglerae* seedlings that were planted in each of the years, the number surviving after one, two, and three years post-transplantation, and the total number surviving after each of the time periods.

Year planted	no. of seedlings planted	no. of surviving seedlings after 1 year	no. of surviving seedlings after 2 years[1]	no. of surviving seedlings after 3 years[1]
2020	64	46	43	34
2021	64	62	55	NA
2022	64	50	NA	NA
Total	192	158	98	34

[1] Seedling survival was not assessed in the same year that they were planted, hence the NA values.

(non-cumulative data). Cluster identity (1 – 4) and site identity (Grootkloof or Castle Gorge) were included as random effects to account for spatial structure in the data, with cluster nested within site. All other predictor variables were included as fixed effects. Year planted was not included in the analyses of survival after three years, as only one cohort of seedlings were monitored for this time period (i.e. only seedlings planted in 2020 formed part of this dataset). These analyses of survival were repeated on a non-cumulative basis (survival in specific years), but yielded very similar results to the cumulative data (Tables A1 and A2). Thus, only the results from the models using the cumulative data are discussed.

The effect of the following predictor variables on seedling growth one, two, and three years after being transplanted were tested: light exposure, fire exposure, PS treatment, and year planted. The growth of the seedlings was determined by calculating the difference between the volume at each of the above time periods and the initial volumes when planted. None of the predictor variables were strongly collinear (VIF <3; Tables A5 and A7).

The analyses of growth were conducted using GLMMs with a gaussian distribution. Site and cluster identity were included as random effects to account for spatial structure in the data, with cluster nested within site. All other predictor variables were included as fixed effects. Year planted was again not included in the analyses of growth after three years. These analyses of growth were repeated on a non-cumulative basis (Tables A1 and A2), but also yielded very similar results to the cumulative data. As in the analyses of survival, only the results from the models using the cumulative data are discussed.

All statistical analyses were performed in R version 4.1.0 (R Core Team, 2021) with additional functions from the car (Fox & Weisberg, 2019), ggplot2 (Wickham, 2016), corrplot (Wei et al., 2017), glmmTMB (Brooks et al., 2017), MuMin (Barton, 2012), scicio (Pedersen & Crameri, 2020) and emmeans (Lenth, 2024) libraries.

3. Results

3.1. Survival

At the conclusion of this study, 72 % of the transplanted seedlings were still alive, with varying survival rates for each of the cohorts (Table 1). The shade treatment and the transplantation of larger seedlings yielded the highest survival rates. Seedling survival one year post-translocation was significantly ($p < 0.05$) higher in shaded microsites (Estimate = −1.73; 95 % CI: [−2.69, −0.77]) and for larger seedlings (Estimate = 0.097; 95 % CI: [0.004, 0.20]), (Table 2, Fig. 3, Tables A1 and A2). Survival two and three years after planting was only significantly related with light exposure (Estimate = 1.61; 95 % CI: [0.61, 2.62] and Estimate = −2.33; 95 % CI: [−3.64, −1.01] respectively), with higher survival in shaded microsites (Table 2, Fig. 3, Tables A1 and A2).

3.2. Growth

After one year post-translocation, 82 % of the seedlings were still alive, with these individuals then used in the analyses of growth. Seedlings that were planted in 2021 grew more than the seedlings planted in 2020 and 2022. Seedlings that received PS grew more than those that did not. In contrast, seedlings that were exposed to fire grew less (and in some cases had negative growth) than those that were not. Seedling size two and three years after planting was not significantly related to any of the predictor variables.

Seedling growth one year post-translocation was significantly related to year planted, with seedlings planted in 2021 obtaining the highest change in mean volume (Table 2, Fig. 4a, Tables A1 and A2). Seedling growth one year post-translocation was significantly related to PS treatment, with seedlings treated with PS obtaining the highest change in mean volume (Estimate = 6.74; 95 % CI: [0.64, 12.84]), (Table 2, Fig. 4b, Tables A1 and A2). Seedling growth one year post-translocation was significantly related to fire exposure, with seedlings that were not burnt obtaining the highest change in mean volume (Estimate = −10.92; 95 % CI: [−19.89, −1.96]), (Table 2, Fig. 4c, Tables A1 and A2).

A post-hoc analysis using Tukey's Honest Significant Difference (HSD) test was conducted to compare the differences between the various years that aloes were planted and their effect on growth. These results indicated that 2021 was significantly different from 2020 and 2022, with no significant difference between 2020 and 2022 (Table A8).

4. Discussion and conclusions

The results of our study regarding treatments and factors affecting the survival and growth of translocated *A. peglerae* seedlings are encouraging, and if applied to larger-scale translocations may increase the chance of long-term success. These treatments and factors, together

Table 2
Results from generalized linear mixed models for survival and growth of *Aloe peglerae* seedlings one, two, and three years after being transplanted. $n_{Survival}$ = the number of seedlings that were initially planted and could potentially have survived 1-, 2- or 3-years post-translocation; $n_{Growth\ rate}$ = the number of seedlings across the three planting years that survived and for which the growth rates could be measured (see Table 1 for details).

					Fixed effect coefficients[1]				
Response variable	Time post-translocation	n*	Model conditional R^2 (%)	Model p-value	Year planted	Light exposure	PS treatment	Fire exposure	Starting volume[2]
Survival	1 year	192	49.4	**<0.001**	2021> 2022> 2020	**Shade > Sun**	NT > PS	Yes > No	**0.097**
	2 years	128	30.7	**<0.001**	2020 > 2021	**Shade > Sun**	PS > NT	No > Yes	−0.096
	3 years	64	43.3	**<0.001**	NA	**Shade > Sun**	PS > NT	Yes > No	0.053
Growth rate	1 year	158	28.9	**<0.001**	**2021> 2020 = 2022**	Shade > Sun	**PS > NT**	**No > Yes**	NA
	2 years	98	22.7	0.335	2020> 2021	Sun > Shade	PS > NT	No > Yes	NA
	3 years	34	8.4	0.568	NA	Sun > Shade	PS > NT	No > Yes	NA

* Only surviving seedlings were used to calculate the seedling growth, thus, the number of seedlings included in the analyses of growth is divergent from the number of seedlings included in the analyses of survival.

[1] Categorical predictors are ranked according to their coefficients, with significant predictors indicated in bold. PS= Potassium silicate applied to aloes. NT= No Potassium silicate applied to the aloes. Shade = aloes shaded by a grass tuft. Sun = aloes in full sunlight.

[2] Starting volume was used to calculate the seedling growth, thus, it is not included in the results of the analyses of growth.

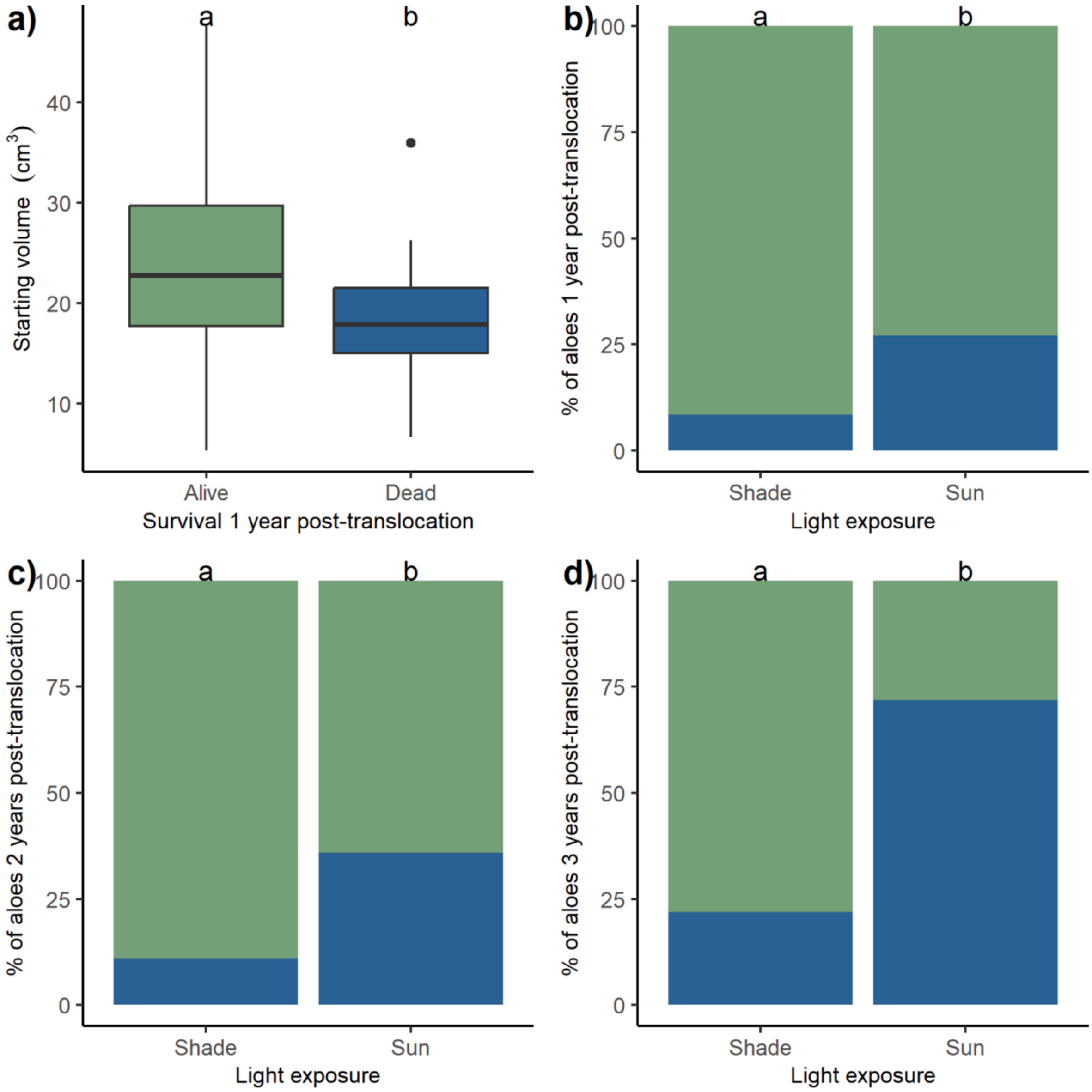

Fig. 3. Surviving seedlings (green) and seedlings that died (blue) a) one year post-translocation compared to their mean starting volumes (n = 192), b) one year post-translocation compared to light exposure and represented as a percentage (n = 192), c) two years post-translocation compared to light exposure (n = 128), and d) three years post-translocation compared to light exposure (n = 64). Significant differences are indicated on box plots and bar graphs with different letters. In a) the extent of the boxes indicates the upper and lower quartiles with the median indicated by the horizontal black line. The whiskers indicate the maximum and minimum values, and the black dots indicate outliers.

with their implications, are discussed below.

4.1. *The provision of shade*

Shading had the most consistent effects on the survival rates of the translocated aloes, with starting volume also influencing survival during the first year post-transplantation. For all periods of observation, aloes planted in the shade of a grass had significantly higher survival than those planted in full sun. This effect is well documented in the literature, and referred to as the nurse plant theory (Brooker et al., 2008; Callaway, 1995). Nurse plants provide a variety of benefits to target plant species (Ren et al., 2008) primarily by providing conditions more favourable than the surrounding environments, such as reduced light intensity (Valiente-Banuet & Ezcurra, 1991), lower temperatures in summer (Godínez-Álvarez et al., 2003), higher temperatures in winter (Duker et al., 2015), higher soil humidity and nutrient content (Muro-Pérez et al., 2012), and herbivore avoidance (Padilla & Pugnaire, 2006). The benefits provided to the transplanted seedlings in the shade of grasses could be related to any of the above. Indeed, facilitation effects have been identified between nurse plants and various succulents in the Asphodelaceae (Dortort, 2024; Duncan et al., 2005; Molteno et al., 2017; Titus et al., 2012; van Blerk, 2013). The benefits of shading from nurse plants have also been noted in several other conservation translocation studies (Elliott & Turner, 2021; Menges et al., 2016; Ren et al., 2008).

Recruitment rates in habitat are very low (personal observation), with the few observable seedlings (<5) all occurring in either the shade of a grass or sedge. As mature *A. peglerae* grow in full sun, this suggests that in time the aloe seedlings may outcompete the nurse plant, as seen in other succulent-nurse plant interactions (Armas & Pugnaire, 2005), or alternatively, they might outlive the nurse plant (Sosa & Fleming, 2002). Such interactions between succulents and their non-succulent nurse plants have been noted elsewhere in the world (Flores-Martínez et al., 1998; Flores-Torres et al., 2019). Our results demonstrate that future *A. peglerae* conservation translocations will benefit by the appropriate

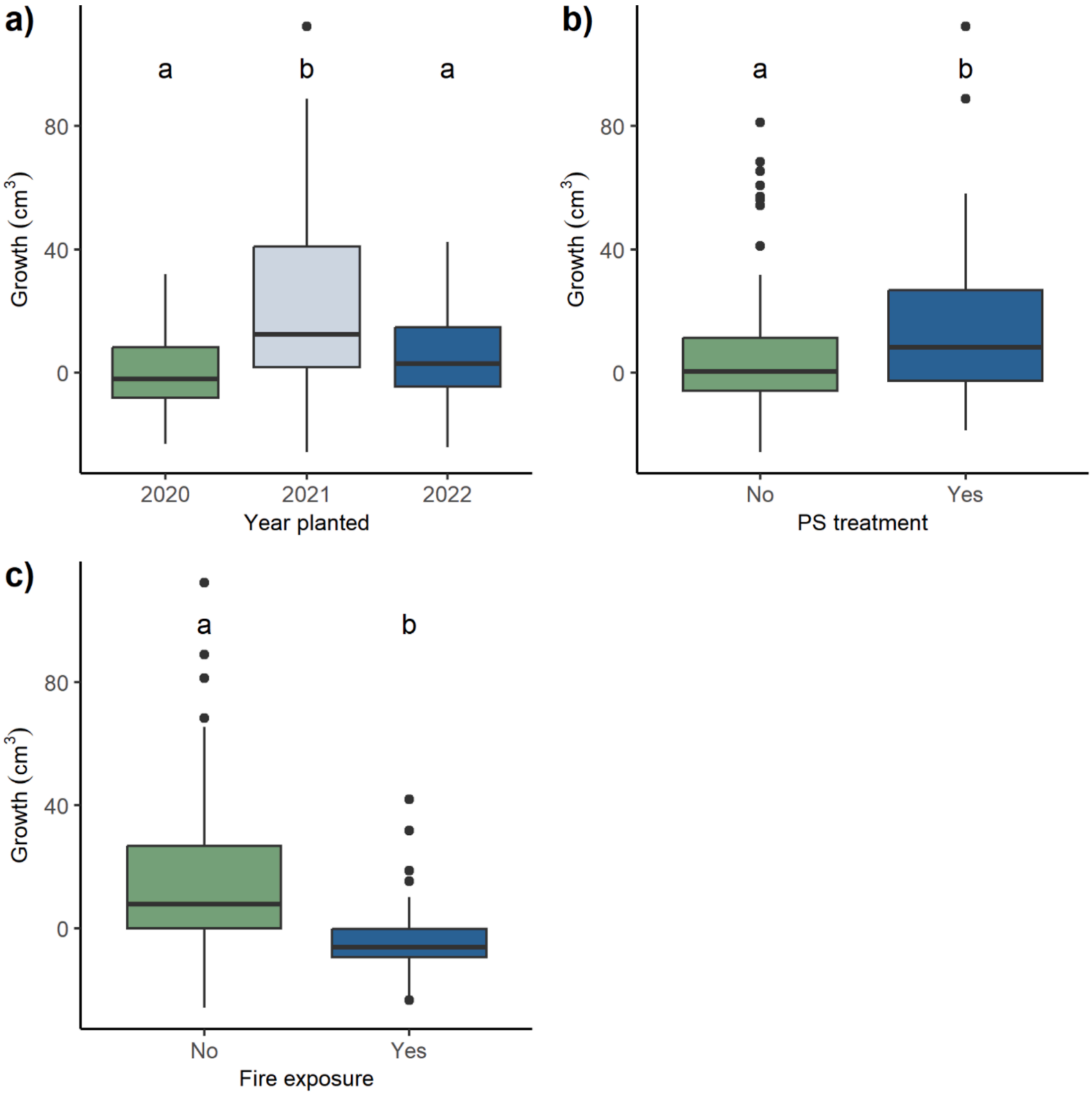

Fig. 4. The relationships between seedling growth one year post-translocation (n = 158) compared to a) the year seedlings were planted, b) PS application, and c) fire exposure. Significant differences are indicated on box plots with different letters, whereas boxes with identical letters do not differ significantly from each other, according to a Tukey's post-hoc test. The extent of the boxes indicates the upper and lower quartiles with the median indicated by the horizontal black line. The whiskers indicate the maximum and minimum values, and the black dots indicate outliers.

use of shading provided by nurse plants.

4.2. Seedling size

Larger seedlings had higher survival rates, as they are likely more resilient to harsh environmental conditions, primarily through greater resource availability in the form of moisture content and energy reserves, which would increase their ability to survive drought and other environmental stressors. Similar results were obtained in other conservation translocation and restoration efforts (Albrecht & Maschinski, 2012; Godefroid et al., 2011; Grossnickle & Macdonald, 2018; Guerrant, 2012; Wendelberger et al., 2008). Our findings suggest that seedlings should be as large as possible before being transplanted, and thus, the propagation phase should be long enough for the seedlings reach a certain minimum size (Commander et al., 2018).

4.3. Climatic variation

Aloes planted in 2021 had higher growth rates than those planted in other years, which may potentially reflect the much higher rainfall in the area during 2021 (629.5 mm), compared to 2020 (385.9 mm) and 2022 (467.2 mm) (South African Weather Service, 2019). Such trends were also observed in other conservation translocation studies (Cypher et al., 2014; Dillon et al., 2018; Janissen et al., 2021; Maschinski et al., 2004; Monks et al., 2023), where favourable years resulted in better growth. However, data from more years and/or experimental rainfall manipulations would still be required to confidently attribute inter-annual variation in climate to variation in *A. peglerae* performance. Additionally, the practical implications of such findings for conservation translocations are challenging to implement, owing to the inherently stochastic nature of rainfall patterns (Koutsoyiannis & Pachakis, 1996). However, our results indicate that broader predicted rainfall patterns should be considered before undertaking future conservation translocations, including delaying translocations to coincide with higher rainfall periods (Jusaitis, 2005) linked to the El Niño Southern Oscillation cycle (Cane, 2005; Nicholson & Selato, 2000).

4.4. Exposure to fire

Fire exposure did not have a significant effect on the survival of the aloes, highlighting a high level of fire tolerance, even in smaller individuals. Indeed, various aloe species have mechanisms that allow tolerance of fire, including the protection of the apical meristem within their robust outer leaves (Cousins & Witkowski, 2012; Kativu et al., 2019). However, it was somewhat unexpected that such young seedlings would have the leaf matter required to protect themselves from fire. As *A. peglerae* typically occurs in very rock microsites, it has been hypothesized that the high rock cover results in a fire avoidance ability of the species (Arena et al., 2015). Our observations show that high rock cover did not exclude fire from *A. peglerae* microsites, but may reduce fire intensity due to the lower fuel loads (Arena et al., 2015). The architecture of *A. peglerae* likely also influences the species' ability to tolerate fire, as it has a low surface area to volume ratio (Bond & van Wilgen, 1996; Montgomery & Cheo, 1971), making it less susceptible to severe fire damage than, for example, grasses. Additionally, the succulent nature and associated high water content (Bond & van Wilgen, 1996; Xanthopoulos & Wakimoto, 1993) of the species likely further reduces its susceptibility to fire damage.

Although the survival of the aloes was not affected by fire exposure, fire did have a significantly negative effect on the growth of the seedlings, but only during the first year post-translocation. Even though seedlings may survive fire, damage to their outer leaves reduces the plants' biomass and volume. The resulting loss of stored resources and ability to photosynthesise is then likely greater (in relative terms) than in older plants which have greater total reserves and photosynthetic capacity. These findings are in line with other studies where younger *Kumara plicatilis* (L.) G. D. Rowley (Asphodelaceae) plants were more adversely affected by fire exposure than older individuals (Cousins et al., 2016). The implications of our findings are that fire should be suppressed in conservation translocation sites, when possible, particularly during the first year post-translocation.

4.5. Potassium silicate application

PS application had a significantly positive effect on the growth of the seedlings one year post-translocation. Although PS was used in this study with the intent of increasing the drought tolerance of the treated seedlings, it is unclear which of the benefits of exogenous PS resulted in the observed increased growth. However, other authors have detected similar positive effects on the growth of *Aloe vera* following PS application (Xu et al., 2015). Regardless of the mechanisms involved, exogenous PS application was shown to be a novel and beneficial addition to conservation translocation efforts that should be considered in future studies.

4.6. Implications for future work

Besides sun exposure, none of the other predictor variables affected either the growth or survival of the aloes after more than one year post-translocation. This suggests that future larger-scale translocation efforts (in all appropriate categories of conservation translocations (IUCN/SSC, 2013)) with this species should primarily focus on planting seedlings in shaded microsites. Other biotic and abiotic factors, including seedling size when planted, fire exposure, climatic variation, and biostimulant application, should still be considered to maximise survival and growth rates in the crucial first year of establishment post-translocation. Additionally, collecting data across more years would be necessary to identify which environmental conditions (e.g. annual rainfall) are driving inter-annual differences in growth.

There are several other aspects of conservation translocations on *A. peglerae* that future studies should investigate, including the optimal period of propagation prior to translocation, the optimal PS application rates, the effect of varied levels of shade, the fire frequency that promotes seedling survival, and the long-term trends of survival and growth. The relatively low explanatory power of some of the models suggest that other unmeasured variables may be affecting the outcomes of our experiments, and that future conservation translocations should attempt to identify and manipulate these variables. Crucially, the genetic diversity of *A. peglerae* should also be investigated across its distribution range to both enable the identification of appropriate donor populations for translocations (see e.g. Dalrymple & Broome, 2010), and to determine if low genetic diversity exists within the species and/or certain populations (Ducrettet et al., 2022). Additionally, future translocations should aim to maximise the number of propagule source individuals to improve the short-term survival of translocated individuals (Schäfer et al., 2020), and their persistence (Encinas-Viso & Schmidt-Lebuhn, 2018; Godefroid et al., 2016). Interactions of the transplanted seedlings with soil microbiomes should also be investigated, as other studies have found beneficial associations between soil microbes and conservation translocations of threatened plants (see e.g. Doyle et al., 2021; Haskins & Pence, 2012). Finally, following a translocation, monitoring should continue for as long as possible before conclusions regarding the level of success can be determined (Bellis et al., 2024; Commander et al., 2018; Godefroid et al., 2011; Julien et al., 2023; Monks et al., 2012).

This study demonstrates the need for conservation translocation protocols based on empirical data, and that valuable information can be obtained by conducting such studies prior to larger-scale efforts, which can optimize success rates and funding utilisation (Commander et al., 2018). Future efforts should aim to publish strict protocols whilst making the associated data available to other researchers (Doyle et al., 2023). Considering the large number of threatened succulents, more similar studies should be conducted to inform future conservation efforts, particularly given the lack of conservation translocation protocols for threatened succulent species, both in South Africa and abroad. Based on our findings, a suggested protocol for succulent conservation translocations is provided to stimulate further research (Appendix B).

CRediT authorship contribution statement

A.W. Frisby: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **M. Momberg:** Writing – review & editing, Project administration, Methodology, Investigation, Conceptualization. **P.C. le Roux:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Arnold Frisby reports financial support was provided by Botanical Education Trust (South Africa). Arnold Frisby reports financial support was provided by Mountain Club of South Africa. Arnold Frisby reports financial support was provided by Botanical Society of South Africa. Arnold Frisby reports financial support was provided by Richard Watmough Magaliesberg Conservation Fund. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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undertaking of this study on their properties.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnc.2025.126851.

Data availability

Data will be made available on request.

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