Restoring South African subtropical succulent thicket using *Portulacaria afra*: root development of cuttings varies amongst parent-plants and harvest site

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The restoration of succulent thicket (the semi-arid components of the Albany Subtropical Thicket biome endemic to South Africa) has largely focused on the reintroduction of *Portulacaria afra* L. Jacq — a succulent shrub — through the planting of unrooted cuttings directly into field sites. However, there has been inconsistent establishment and survival rates, possibly due to the poor condition of source material used. Here we test the effect of parent-plants and harvesting site on the root development of *P. afra* cuttings. Ten sites were selected along a ~110 km transect, with cuttings harvested from five parent-plants per site. Leaf moisture content was determined for each parent-plant at the time of harvesting. Root development — percentage of rooted cuttings and mean root dry weight — was evaluated for a subset of cuttings from each parent-plant after 35, 42, 48, 56, and 103 days after planting in a common garden setting. We found evidence for the percentage of rooted cuttings to be strongly associated with harvesting site across all sampling days (p <0.005 for all ANOVAs). Additionally, nested ANOVAs revealed strong evidence for site and individual plant identity affecting the root dry weight for all sampling days (p <0.0001). These differences are likely a consequence of some underlying physiological factors; however, leaf moisture content of the parent-plant at the time of harvesting was a poor predictor of rooting potential. Our findings suggest that parent-plant condition plays a crucial role in root development and that this may be a critical component that needs to be understood as part of any restoration programme. Further work is required to identify optimal environmental conditions that may promote or impede root development in *P. afra* cuttings.

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18 ## Abstract

19 The restoration of succulent thicket (the semi-arid components of the Albany Subtropical Thicket biome
20 endemic to South Africa) has largely focused on the reintroduction of *Portulacaria afra* L. Jacq — a
21 succulent shrub — through the planting of unrooted cuttings directly into field sites. However, there has
22 been inconsistent establishment and survival rates, possibly due to the poor condition of source material
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27 Leaf moisture content was determined for each parent-plant at the time of harvesting. Root development
28 — percentage of rooted cuttings and mean root dry weight — was evaluated for a subset of cuttings from
29 each parent-plant after 35, 42, 48, 56, and 103 days after planting in a common garden setting.

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31 We found evidence for the percentage of rooted cuttings to be strongly associated with harvesting site
32 across all sampling days ($p < 0.005$ for all ANOVAs). Additionally, nested ANOVAs revealed strong
33 evidence for site and individual plant identity affecting the root dry weight for all sampling days ($p
34 < 0.0001$). These differences are likely a consequence of some underlying physiological factors; however,
35 leaf moisture content of the parent-plant at the time of harvesting was a poor predictor of rooting
36 potential. Our findings suggest that parent-plant condition plays a crucial role in root development and
37 that this may be a critical component that needs to be understood as part of any restoration programme.
38 Further work is required to identify optimal environmental conditions that may promote or impede root
39 development in *P. afra* cuttings.

Introduction

Arid and semi-arid (dryland) ecosystems are characterised by low and, in certain systems, unpredictable rainfall, which can cause considerable stress to plants. The introduction of additional, prolonged and compounding stressors, such as non-native herbivores, to these environments often compromises ecosystem functioning and triggers a transition to an alternate, degraded state (Verwijmeren et al., 2013; Scheffer et al., 2001). Restoration of these drylands requires the reversal of these transitions and, ultimately, the reintroduction of the ecosystem processes that have been lost (James et al., 2013). This study focuses on the succulent component of the Albany Subtropical Thicket biome, which occurs in the semi-arid southern coastal lowlands of South Africa, and has been extensively degraded with large-scale restoration initiatives underway.

The degradation of succulent thicket (i.e. arid and valley thicket *sensu* Vlok et al., 2003) serves as a notable example of this transition between states in a dryland ecosystem. By 2002, up to 80% of the ecosystem had been degraded to some extent (Lloyd et al., 2002). The unsustainable browsing of livestock within these succulent thickets has resulted in the transition from a dense, impenetrable closed-canopy system (typically dominated by *Portulacaria afra* Jacq., a succulent tree/shrub endemic to southern Africa) to an open pseudo-savanna. This degraded habitat is typically characterised by an open matrix of bare soil, dwarf shrubs, and ephemeral herbs and grasses, with a few remnant canopy-dominant tree species, such as *Pappea capensis* Eckl. & Zeyh. (Lechmere-Oertel et al., 2008). This loss of plant cover, including *P. afra*, results in the disruption of various ecological processes. Exposed soils are especially prone to: erosion due to higher rates of water runoff (Mills and de Wet, 2019; van Luijk et al., 2013; Cowling and Mills, 2011); loss of soil organic carbon (Mills and de Wet, 2019; Lechmere-Oertel et al., 2008); and, a disruption of soil microbial communities (Schagen et al., 2021). Furthermore, the loss of the cool, damp understory microclimate required for germination (Wilman et al., 2014; Sigwela et al., 2009) halts woody species recruitment in the degraded landscape (Lechmere-Oertel et al., 2005).

The restoration of succulent thicket focuses predominantly on the active planting of unrooted *P. afra* cuttings to facilitate the return of ecosystem functioning. Where successful, this practice has regenerated soil organic carbon (Mills and Cowling, 2006), promoted the return of microbial communities (Schagen et al., 2021), and facilitated the return of natural recruitment dynamics (Galuszynski, 2023; van der Vyver et al., 2013). However, this restoration has failed to produce consistent results, with large-scale planting initiatives in the Great Fish River Nature Reserve and Addo Elephant National Park (representing ~21.3 million planted *P. afra* cuttings) reporting a mean survival of 28% with high variation across sites (Mills and Robson, 2017). However, all the cuttings used in these plantings were sourced from local stands of *P. afra*, likely encompassing a wide range of spatial and temporal environmental conditions. This approach did not consider the potential influence of parent-plant condition on the outcome of restoration initiatives. The condition of parent-plants at the time of harvesting may significantly influenced cutting establishment rates and survival; here we explore the variation in rooting success amongst parent-plants and sites.

Restoration programs are often characterised by low success rates, with a global estimated average of 52% survival (reviewed by Godefroid et al., 2011), which is notably higher than that reported in the *P. afra* reintroduction programs. Low survival has been elsewhere attributed to the poor condition of the plant material used in planting programs (World Agroforestry Centre et al., 2020; Kildisheva et al., 2017),

84 resulting in propagules that are unlikely to tolerate local environmental stresses (Godefroid et al., 2011).
85 In restoration programs around the world, the practice of sourcing propagation material from healthy
86 parent individuals (Kildisheva et al., 2017; Amri et al., 2010; Husen and Pal, 2007; Chalupa, 1993) and
87 populations is recommended (reviewed in Bucharova et al., 2017; Houde et al., 2015; van Andel, 1998).
88 However, this practice is not currently taken into consideration when utilising *P. afra* for restoration
89 purposes.
90
91 Succulent thicket is distributed across a diverse range of environmental conditions, encompassing various
92 drainage basins, elevations, and soil types, as well as differences in timing and amount of rainfall (Mills et
93 al., 2011; Vlok et al., 2003). Rainfall is highly variable at regional to local scales, including frequent and
94 often prolonged droughts that continue for many years, and may include months with little to no rainfall
95 (Archer et al., 2022; Mahlalela et al., 2020; Palmer et al., 2020). We suspect that the physiological
96 condition of *P. afra* at the time of harvesting, which is dependent on the prevailing conditions (Bews and
97 Vanderplank, 1930), may strongly contribute to the likelihood of establishment and subsequent survival
98 of planted cuttings. Parent-plant condition may have contributed towards the highly variable survival in
99 large-scale plantings (e.g., 0–100% reported in van der Vyver et al., 2021a).
100
101 To improve our understanding of the potential effects of parent-plant condition on succulent thicket
102 restoration success, this work quantifies root development in a common garden experiment using cuttings
103 sampled from sites across an environmental gradient. We consider the rate of root development as a
104 crucial determinant of cutting establishment and survival. The experiment uses *P. afra* cuttings obtained
105 from ten sites located along a transect of ~110 km. These sites encompass a diverse range of
106 environmental conditions, including different elevations (190-635 m), and span from a more inland arid
107 northern climate to a more mesic near-coastal climate in the south. Our results demonstrate that there are
108 significant differences in rooting across the various harvesting sites when grown in a common garden,
109 which we attribute to differences in parent-plant condition. These results should serve as important
110 guidelines to future restoration programs.
111

Methods

Recent climatic conditions

114 The Eastern Cape of South Africa experienced a severe and prolonged drought starting in 2015 (Archer et
115 al., 2022; Mahlalela et al., 2020) and continued up to the sampling period of this experiment (October
116 2021, Figures 1, S1, Table S1). This included below average rainfall across all peak rainfall periods over
117 much of the Eastern Cape region, including some of the driest winters recorded since 1981 in some areas
118 along the western interior limit of the region (Archer et al., 2022) (Figure 1). Seasonal greenness (used as
119 a proxy for plant productivity and condition) was lower than pre-drought conditions for winter and
120 summer (Archer et al., 2022), suggesting that plant condition was generally poor across the region,
121 including along the transect from which the *P. afra* was harvested for this study. However, regional
122 patterns are a poor reflection of localised vegetation condition, and thus, to characterise the drought
123 within the study area, precipitation data was extracted from the *CHIRPS* archive (Funk et al., 2015) — an
124 extrapolated rainfall dataset spanning 1981 until the present at a 0.05 arc-degrees resolution generated and
125 hosted by the Climate Hazards Center — using the library *chirps* (version 0.1.4) in R (version 4.2.2; R
126 Core Team, 2022). The monthly rainfall estimates from the CHIRPS data at the various sites sampled
127 along the transect (described above) demonstrated that rainfall was below the monthly average for 14 of

128   the 17 months prior to harvesting of the plant material used in the experiment (Figure 1, S1, Table S1). In
129   addition, in the preceding eight months, the 12-month standardized precipitation index (spi) was lower
130   than -1, shifting between moderately to severely dry conditions; spi was calculated using *SDI* function of
131   the *drought* library (version 1.1; Hao and Zu, 2022)
132
133   *Site selection and sampling*
134   Ten sites were sampled along a transect spanning ~110 km in a north-east to south-west orientation
135   (Figure 2), representing three distinct positions in the landscape: inland mountains (sites 1,2,5, and 6),
136   inland lowlands (sites 3,4,7, and 8) and coastal hills (sites 9 and 10). The rainfall gradient found along the
137   transect is steep due to orographically-induced precipitation due to the Cape mountain chains combined
138   with strong rain shadow effects of extended flat basins between the mountains (Figures 2, S2). Thus, sites
139   in the inland mountains received more rain than neighbouring sites located in the extended rain shadow of
140   the inland lowlands, whereas sites in the coastal hills received the highest rainfall due to low
141   continentality.
142
143   Although there are notable morphological differences across the distribution of *P. afra* (Van Jaarsveld
144   and Le Roux, 2021), no obvious differences were observed in leaf size or plant shape across the transect,
145   which spans a historically continuous distribution of succulent thicket (i.e. we do not consider these to be
146   different or isolated populations). Five individuals were selected from each site, and 42 cuttings were
147   harvested from each individual; note that site 5 only had four individuals (due to the loss of one bag
148   during transport). Plants were selected from areas in the landscape that were considered free from
149   herbivores, e.g. within the road reserve (but in an elevated position unaffected by the road) or within
150   fenced-off experimental plots (sites 3 and 4; which were ~11 years old plants planted as part of the
151   Thicket-Wide Plot experiment, Mills et al., 2015). However, sites 7 and 8 were within a game farm
152   exposed to herbivores, but all cuttings were harvested from large plants above 1.5 m, thus limiting the
153   range of herbivores those branches were exposed to (i.e. only to Greater Kudu). Cuttings were harvested
154   and stored in clear plastic bags and transported back to the laboratory where they were kept at room
155   temperature for four days while being processed. To obtain an estimate of leaf moisture content for each
156   parent-plant, two batches of 30 leaves (per plant) were removed from each parent-plant on the day of
157   harvesting and placed into sealed containers of known weight. These were then reweighed in the lab to
158   obtain the wet weight, and all leaves were dried at 70°C for three days before being weighed to determine
159   leaf moisture content; moisture content was calculated as percentage of wet weight. All parent-plants
160   were harvested on the 13th of October 2021, and the collection permit was obtained from the Eastern
161   Cape Department of Economic Development, Environmental Affairs and Tourism (permit number:
162   HO_RSH_32_2021).
163
164   *Common garden*
165   Cuttings were individually planted into polypropylene UV-protected plastic seed trays (98-cavity trays
166   with volume of ~90 cm^3 per cavity); in total, 22 trays were used. Long cuttings were trimmed to ~20 cm
167   in length. The mean stem diameter of the cuttings was 4.99±0.85 mm and length was 16.9±1.90 cm. The
168   placement of cuttings from harvesting sites and parent-plants was semi-randomised across the seed trays:
169   cuttings from the same individuals were grouped into batches of seven — the number of cavities per
170   column in the seed tray — and the batches were randomised, in lines, across the trays (i.e. cuttings in

171 columns were randomised). This batch randomisation was required as a fully randomised design was
172 logistically not feasible due to excessive handling time.

173

174 The trays were placed in a grid layout into a common garden enclosure where they would receive full
175 sunlight. Trays were spaced 30 cm apart and trays were moved to a new randomly assigned position in
176 the grid on a weekly basis. Trays were watered on a weekly basis if there had been no rainfall during the
177 prior seven days.

178

179 Destructive sampling of a subset of six cuttings per parent-plant took place across seven separate events,
180 specifically 20, 27, 35, 42, 48, 56, and 103 days after harvesting (days 20 to 48 were in November 2021,
181 day 56 in December 2021, and day 103 in January 2022). During each sampling day, the cuttings from
182 each parent-plant were randomly selected from across all trays. Note that due to handling errors, on five
183 instances across the harvesting events the number of cuttings per plant dropped to four.

184

185 On each sampling, the presence or absence of roots was evaluated for the randomly selected cuttings, and
186 roots were also harvested for weighing from sampling day 35 onwards. Soil was gently removed from the
187 roots, and the roots were then dried at 70°C for three days and weighed to four decimal places.

188

189 *Data analyses*
190 The statistical analyses below were conducted in R version 4.2.2 (R Core Team, 2022). The percentage of
191 rooted cuttings was calculated for each parent-plant at each sampling event (i.e. from 5 to 7 cuttings per
192 plant). The percentage values were arcsine transformed and significant differences in rooting, per
193 sampling event, and amongst sites were tested using ANOVAs and post-hoc Tukey tests. These analyses
194 were conducted using the *aov*() and *TukeyHSD*() functions from the *stats* v4.2.2 library (R Core Team,
195 2022). Nested ANOVAs were used to test for significant differences in root weight across plants and
196 sites, with individuals nested within sites for each sampling event. The nested ANOVA was calculated
197 using the *lme()* function of the *nlme* library version 3.1-162 (Pinheiro et al., 2023; Pinheiro and Bates,
198 2000); the formula structure was (RootMass~Site,random=~1|Individual) as suggested by Mangiafico
199 (2015). Post-hoc Tukey tests on each nested ANOVA were conducted using the *glht()* function of the
200 *multcomp* version 1.4-22 library (Hothorn et al., 2008). Note that although the experimental design was to
201 measure five cuttings per plant per harvesting event, this number varied between four and seven due to
202 handling errors. As ANOVAs may be influenced by unbalanced sample sizes, we reduced the dataset to
203 four plants per population and four cuttings per plant and re-ran the nested ANOVA. Significance
204 categories (described below) remained unchanged (see supplementary R code) and so we report the
205 results for the complete dataset. The descriptions of statistical significance are reported following the
206 conventions suggested by Muff et al. (2022). As such, p >0.1 is described as no evidence, p <0.10-0.05 is
207 described as weak evidence of an effect, p <0.05-0.01 is described as moderate evidence of an effect, p
208 <0.01-0.001 is described as strong evidence for an effect, and p <0.001 is described as very strong
209 evidence of an effect.

210

Results

212 Leaf moisture content was found to vary amongst plants within sites and across sites (Figure 2B);
213 however, there was no clear pattern between the leaf moisture content at the time of harvesting and the
214 final mean dry root mass of each parent-plant (Figure S2).

215
216 The results of the ANOVA on the arcsine-transformed rooting percentage data revealed strong evidence
217 for the effect of site across all sampling events (p <0.005 for all ANOVAs; see Table S2 for further
218 details). The percentage of rooted cuttings per plant increased over time. However, this trend varied
219 across individuals and sites (Figure 3). Some sites exhibited high (near 100%) rooting from day 35
220 onwards (e.g., sites 6 & 10), whereas some had improved rooting percentage over time (e.g. sites 1, 7),
221 and others had minor improvements but stayed relatively low (<75%, e.g., sites 4 & 8). In a few cases,
222 cuttings from specific parent-plants had consistently low rooting success (i.e. <40%) across all sampling
223 days (Figure 3).
224
225 We found strong evidence for the effect of site and parent-plant on rooting within all sampling days for
226 both rooting percentage and mean root dry weight: p <0.0001 (all significance values reported in Tables
227 S2 and S3). Furthermore, parent-plants that exhibited rapid root initiation (i.e., half or more of the
228 cuttings had developed roots 20 days after being harvested from the parent-plants) maintained a higher
229 rooting percentage and in most instances higher mean root dry weight after 103 days (Figures 3, 4). It
230 should be noted that sites 3, 4, 5, and 7, which had relatively low percentages of rooted cuttings after 20
231 days (10-20%) were able to reach comparatively high proportions of rooted cuttings per individual after
232 the 103-day experimental period (67-97%; Table S2). However, the delayed rooting of these plants
233 resulted in a lower accumulation of dry root weight over the course of the experiment (Figure 4 and Table
234 S3).
235

Discussion

237 We found strong evidence for differences in rooting amongst individuals and sites, both in terms of the
238 percentage rooting and dry root mass. We will discuss these results first in terms of the local environment
239 and its potential influence on parent-plant condition, and then how variable rooting establishment impacts
240 both restoration initiatives and experiments.
241
242 The parent-plant material for this study was harvested amidst a multi-year drought (Archer et al., 2022;
243 Mahlalela et al., 2020; Figure S1), and drought stress is likely a major contributing factor to varying plant
244 condition and consequently influenced the root development of the cuttings. Despite the regional drought,
245 moisture availability varied across the study transect (Table S1, Figures S1); the coastal hill sites (9 and
246 10) experienced a greater amount of rainfall than the interior sites (1–8) and the inland mountainous sites
247 (1, 2, 5, and 6) had greater rainfall than neighbouring inland lowlands (3, 4, 7, and 8). Excluding sites 1
248 and 2, this is evident in the leaf moisture content measured for parent-plants at each site (Figure 2).
249
250 The physiological consequences of drought stress in *P. afra* include reductions in enzyme activity,
251 decreased leaf chlorophyll content, and a shift towards the dominance of CAM photosynthesis (Guralnick
252 and Ting, 1987). Additionally, the relative ratios of stored carbohydrates and soluble sugars in *P. afra*
253 leaves shift in response to seasonal droughts, with the availability of soluble sugars decreasing in drier
254 winter months (Bews and Vanderplank, 1930). These physiological changes likely contribute to the
255 ability of *P. afra* cuttings to initiate root development.
256
257 The formation of roots in plant cuttings involves the process of cellular redifferentiation, whereby
258 predetermined cells shift their morphogenetic path to an undifferentiated state (mother cells) that can

259 initiate the formation of root primordia (Husen and Pal, 2007; Aeschbacher et al., 1994; Friedman et al.,
260 1979). This process requires an abundance of soluble sugars that provide the energy for protein synthesis
261 and peroxidase activity required for cell division (Husen and Pal, 2007; Bakshi and Husen, 2002). The
262 decreased availability of soluble sugars reported in drought-stressed *P. afra* (Bews and Vanderplank,
263 1930; or overbrowsed plants, discussed later) may be a contributing factor to the poor and highly variable
264 rooting in plants sourced from sites 3, 4, and 8 (Figure 3, Tables S2 and S3). Parent-plant leaf moisture
265 content and estimated received rainfall does not provide a clear pattern regarding root initiation and
266 development. For example, cuttings from sites with high precipitation and had high leaf moisture (>80%;
267 sites 6, 9 and 10) showed rapid root initiation and growth, but so did those from site 2 where leaf moisture
268 was far lower (~70%) as was recent precipitation (Table S1). However, root initiation and growth was
269 depressed across all other sites that had both low leaf moisture (<80%) and estimated recent precipitation.
270 Thus, low leaf moisture content of a parent-plant is not necessarily an indicator of cutting rooting
271 potential. It may not be a reliable indicator because *P. afra* can remain in a drought stressed state for a
272 long period of time, switching from C3 to CAM or CAM-idling photosynthesis (Guralnick and Ting,
273 1986, 1987), with no way to determine the time spent in a low leaf moisture state, and thus the overall
274 condition of the plant.

276 Beyond precipitation, the sites harvested in this study represent a variety of environments, including
277 different physical environments (aspect, elevation, and possibly geology), local climate, and browsing
278 pressures. Below we speculate on a range of factors that may also influence parent-plant condition,
279 specifically dew formation, temperature, edaphic conditions, and herbivory.

281 In addition to topographic effects on rainfall (i.e. mountains vs lowlands), dew formation may also be
282 more prevalent in the mountainous areas compared to the lowlands (Kidron et al., 2000). Dew is an
283 important source of moisture in arid and semi-arid ecosystems (Fan et al., 2023; Jia et al., 2019; Uclés et
284 al., 2014). Foliar absorption of dew has been demonstrated in a variety of *Crassula* (a genus of CAM
285 succulents commonly found in succulent thicket communities) (Martin and Von Willert, 2000). As the
286 quantities of water accumulated during dewfall are relatively low, it is unlikely that it will have long-
287 lasting effects on leaf moisture content, but it may contribute to buffering the physiological responses to
288 drought stress. Thus, foliar dew absorption could potentially explain the high rooting potential of cuttings
289 sourced from mountainous sites with low leaf moisture contents and low rainfall (specifically sites 1 and
290 2; Figure S2). However, foliar dew absorption in succulent thicket plants, including *P. afra*, is yet to be
291 explored.

293 In addition to affecting local moisture regimes, topography greatly impacts local temperatures. In general,
294 the lowlands in the region experience lower minimum temperatures (Duker et al., 2015, 2020), which can
295 place more stress on cold-sensitive plant species (e.g. *P. afra*), reducing the available resources for root
296 development (Fernández et al., 2007). This may, in part, contribute to the poor rooting observed in
297 lowland sites (3, 4, and 8).

299 Local edaphic conditions may also impact parent-plant condition by mediating water and nutrient
300 availability (e.g. Mazaheri and Mahmoodabadi, 2012; Bacon, 2009; Mamedov et al., 2001). Thus, soil
301 may influence parent-plant resilience to drought (Xu et al., 2021; Tariq et al., 2017) and extreme climatic
302 events (Fernández et al., 2007). The soil properties along the transect may be highly variable, with rapid

303   and extreme transitions (e.g. bontveld; Carvalho and Campbell, 2021). Establishing the role of soil on
304   parent-plant condition is beyond the scope of this study.
305
306   Despite efforts to select *P. afra* plants that were unaffected by local browsing pressure, sites 7 and 8 were
307   located within an area that did include herbivores. This may have contributed to the comparatively low
308   starting leaf moisture content of plants sourced from these sites (Figure 2), losing moisture through
309   wounding. Furthermore, the physiological resources required for cellular regeneration (i.e. soluble sugars)
310   may be more limited in these plants as they frequently have to mobilise these resources to respond to
311   herbivore damage. This may have contributed to the variable rates of root development in cuttings
312   sourced from these sites (Figures 3 and 4). We suspect that moderate to high levels of herbivory will have
313   a pronounced negative effect on parent-plant condition, especially during dry phases, and subsequently
314   impact cutting root development.
315
316   Thus, multiple factors or a single factor, may be responsible for the variation in rooting observed in this
317   study. Further research as to the importance of these factors during dry and wet phases is necessary to
318   understand how they impact rooting.
319
320   *Parent-plant condition in restoration initiatives and experiments*
321   The findings presented here (i.e., that source location impacts plant condition and consequently root
322   development in *P. afra* cuttings) have important implications for succulent thicket restoration initiatives.
323   Cutting source location effects have not been considered in *P. afra* planting efforts to date, and as shown
324   here (initially demonstrated in Galuszynski et al., 2023) may play an important role in unrooted cutting
325   establishment and survival, particularly under field and nursery conditions. As previously noted, planting
326   of unrooted *P. afra* cuttings is an unreliable restoration practice (van der Vyver et al., 2021b; Mills and
327   Robson, 2017). A biome-wide field experiment — consisting of 330 plots to test the viability of various
328   planting treatments using unrooted *P. afra* cuttings — revealed that cutting survival was affected by stem
329   diameter (i.e., larger cuttings established more readily than small cuttings), uncontrolled herbivory, and
330   planting into the incorrect habitat (van der Vyver et al., 2021b). However, the reported variability in
331   survival may also be due to the condition of the parent-plants, which has the potential to override any
332   treatment effects (Galuszynski et al., 2023; this study). While cutting size and planting habitat can be
333   easily managed, there is currently no clear method for identifying optimal material to source cuttings for
334   restoration. As leaf moisture content is not a fully suitable proxy for plant condition, we urge restoration
335   practitioners to focus harvesting efforts towards individual plants that exhibit high leaf moisture content
336   and/or fresh recent growth (typically identifiable by smooth red stems >10 cm long), as this provides
337   some indication that the parent-plant has sufficient resources to support active growth. Rooting these
338   cuttings in a nursery setting before field planting can avoid some of the issues associated with source
339   material effects by filtering cuttings that failed to initiate root development due to the parent-plant effect.
340
341   The results from this study support the findings of Galuszynski et al. (2023) that parent-plant identity can
342   have an overriding influence on experimental results. Thus, we urge experimental work conducted on *P.
343   afra* to account for parent-plant effects, track individual plant identity, and include this as a factor in
344   experimental design.
345

Conclusion

Harvesting location and parent-plant identity had a strong effect on the rate of root establishment in a moisture-abundant common garden experiment (versus the arid conditions experienced in inland degraded succulent thicket). As rainfall is often unpredictable and short- and long-term droughts are common in the Eastern Cape, parent-plant condition may have substantially contributed to the highly variable survival rates observed in large-scale restoration and research initiatives using *P. afra*. We find that leaf moisture content is not a sufficiently reliable predictor of root development in unrooted *P. afra* cuttings, but may aid in identifying suitable harvesting sites for restoration initiatives under certain conditions. Practitioners will need to account for the effect of local climate variability on plant condition, and develop systems to address this challenge. Furthermore, future research should include site and parent-plant effects in the experimental design.

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Figure 1

Figure 1

A) The monthly rainfall deviation from the mean monthly rainfall (black dots) for ~18 months prior to harvesting (13 October 2021). B) The 12-month standard precipitation index (spi). Rainfall data extracted from CHIRPS for site 7 (see Fig S1 for the monthly deviation at all sites).
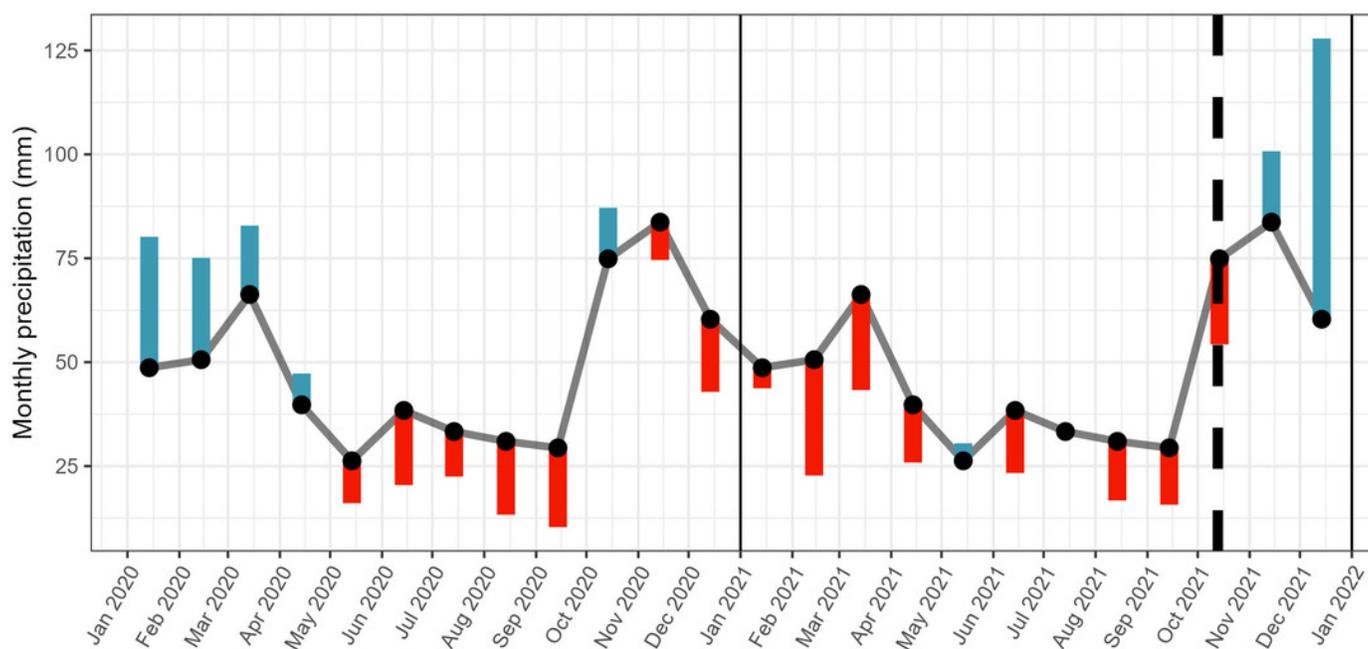
Figure 2

Figure 2

A) A map and elevation profile showing the locations of the ten sites sampled for Portulacaria afra cuttings (Map data © 2023 Google). Cuttings were obtained from five parent-plants per site (except site 5, where only four plants were sampled; see text for details). B) Leaf moisture (percentage of wet weight) of parent-plants harvested at each population.
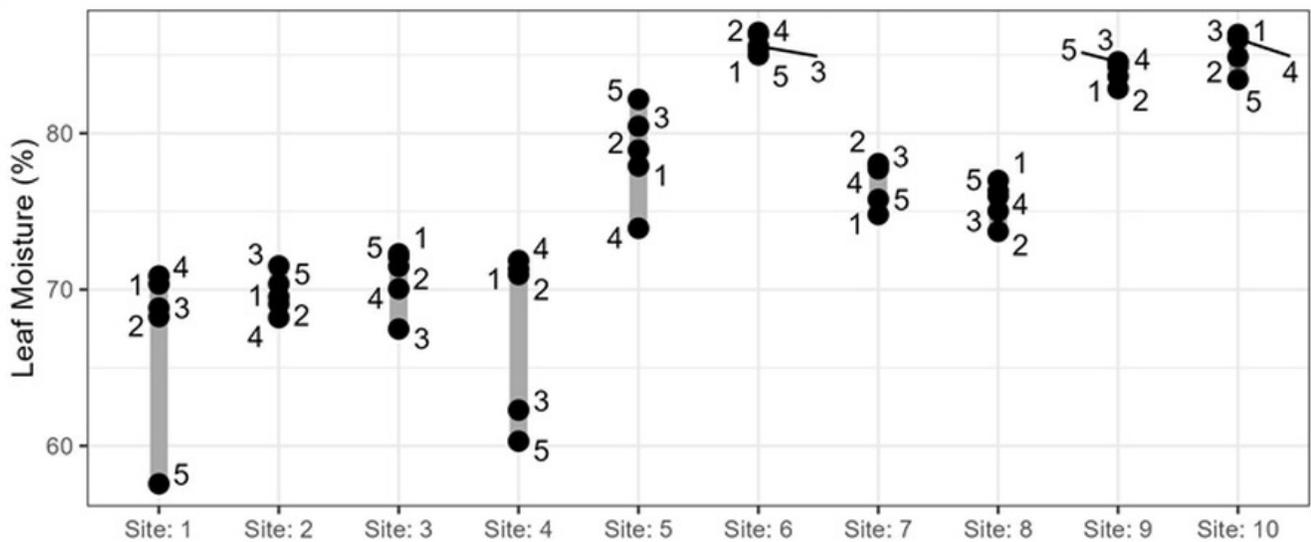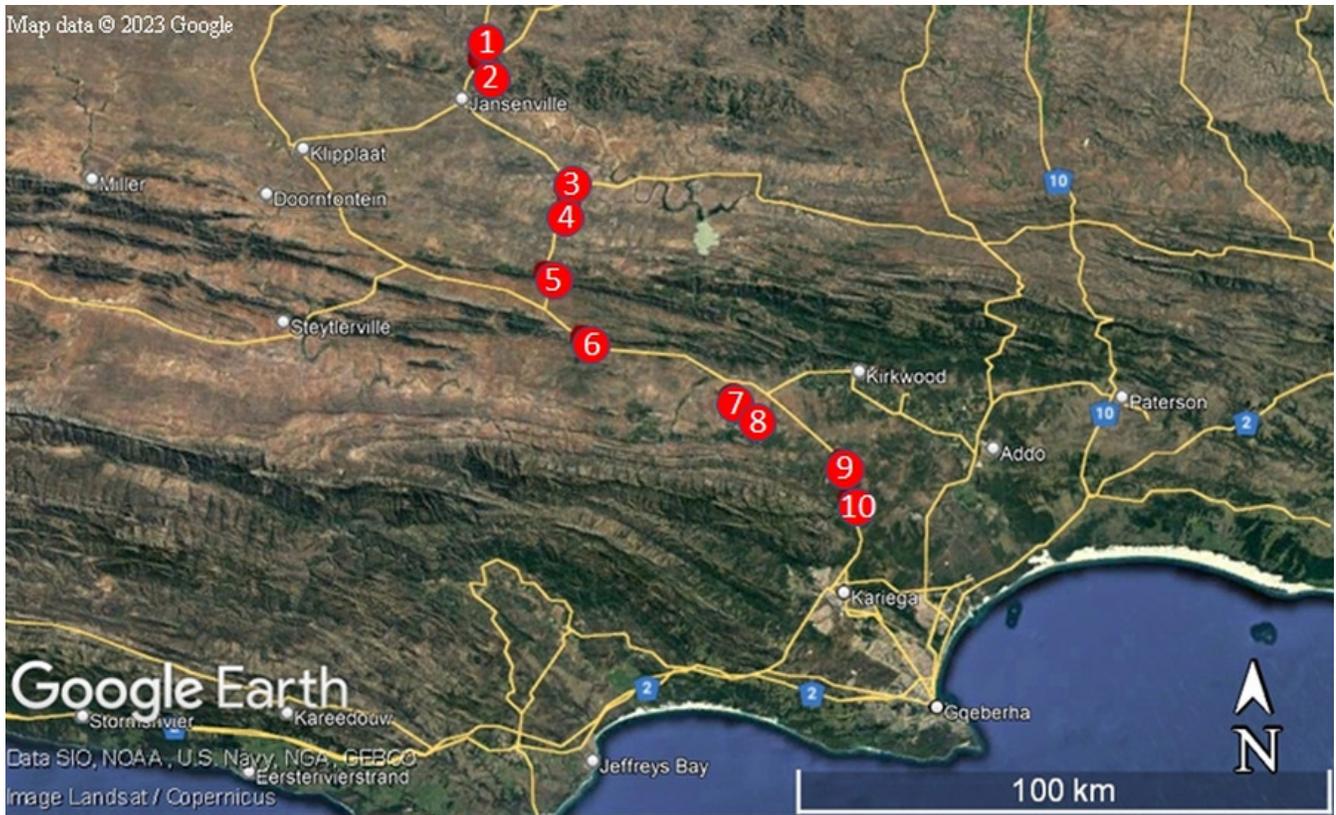
Figure 3

Figure 3

The percentage of rooted cuttings across each population per sampling event: mean (black line) and individual plants (grey lines). See Table S2 for mean and standard deviation of rooting percentages of the six cuttings per plant, and ANOVA and post-hoc Tukey tests. Sites occurred in three landscape positions: inland mountains (1,2,5,6), inland lowlands (3,4,7,8) and coastal hills (9,10).
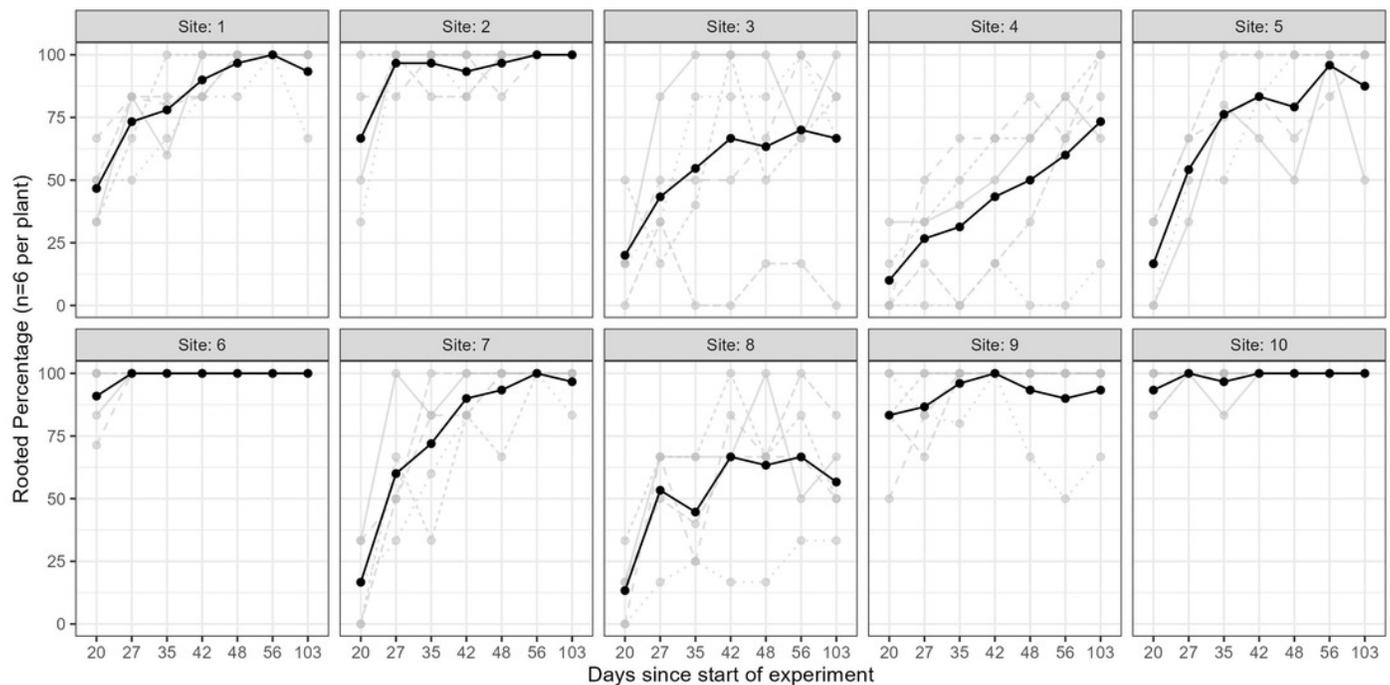
Figure 4

Figure 4

Parent-plant and overall mean dry root weight. Five individual plants were harvested from each site. Mean plant root weight of cuttings from each parent-plant is shown in grey. See Table S3 for mean and standard deviation of dry root weight per plant for each sampling day, and nested ANOVA and post-hoc Tukey tests. Sites occurred in three landscape positions: inland mountains (1,2,5,6), inland lowlands (3,4,7,8) and coastal hills (9,10).