Restoring South African subtropical succulent thicket using *Portulacaria afra*: exploring the rooting window hypothesis

Nicholas C. Galuszynski [1,2], Ryan Forbes[2], Gavin M. Rishworth[3,4], Alastair J. Potts[1,2]

[1] Spekboom Restoration Research Group, Nelson Mandela University, Gqeberha, South Africa
[2] Botany Department, Nelson Mandela University, Gqeberha, South Africa
[3] Institute for Coastal and Marine Research, Nelson Mandela University, Gqeberha, South Africa
[4] Zoology Department, Nelson Mandela University, Gqeberha, South Africa

Corresponding Author:
Alastair J. Potts[1]
Department of Botany, Nelson Mandela University, Gqeberha 6031, South Africa
Email address: alastair.potts@mandela.ac.za

Abstract

Drought prone, arid, and semiarid ecosystems are challenging to restore due to the generally low levels of natural recruitment and survival of reintroduced plants. This is evident in the restoration of degraded habitats in the Albany Thicket Biome (South Africa). The current restoration practise for this ecosystem focuses predominantly on planting *Portulacaria afra* L. Jacq., which is dominant in terms of cover and biomass in intact succulent thicket, but largely absent in regions degraded by domestic livestock. This has been achieved by planting unrooted cuttings with little consideration of soil water availability in a drought-prone ecosystem, which may contribute to the reported variable rates of cutting establishment and survival.

Commented [RLO1]: Suggest you need to include an adjective around 'degraded' as intact systems don't need restoration.
Too many commas here?
Semi-arid or semi-arid?

Commented [RLO2]: practice

Establishment is dictated by successful rooting and this study tests the effects of the timing of water availability after planting on the root development of *P. afra* cuttings. Cuttings were harvested from seven individual plants and grown in a glasshouse setting. From each plant, a total of 84 branchlets were harvested, with twelve per individual used in each watering treatment, resulting in a total of 84 branchlets per treatment. The treatments represented a time-staggered initial watering after planting, including on the day of planting, 4 days, 7 days, 14 days, 21 days, and 28 days after planting. After 32 days, all treatments were watered on a bi-weekly basis for two weeks; a control treatment with no watering throughout the experiment was included. The proportion of rooted cuttings per treatment and dry root mass were determined at the end of the experimental period (day 42). The early onset of watering promoted rooting (H5=11.352, p = 0.045) and had a weak, but non-significant, impact on the final dry root mass (F5,36 = 2.109 p = 0.0631). Unexpectedly, parent-plant identity appeared to have a strong interaction effect on the accumulation of root mass (F36,460 = 5.026, p <0.001; LR7 = 122.99, p < 0.001). The control treatment, which had no water throughout the experiment, had no root development.
These findings suggest that water availability is required for the onset of rooting in *P. afra* cuttings. However, the duration of the experiment was insufficient to detect the point at which *P. afra* cuttings could no longer root once exposed to soil moisture, and thus no rooting window could be defined. Despite harvesting material from the same source population, experiencing the same macro-environmental conditions, parent-plant identity strongly impacted root development — this may explain the variability of cutting establishment and survival in restoration initiatives and experiments. Further work is required to characterise the rooting window, and also to explore the effect of parent plant condition on in-field and experimental restoration results; we urge that experiments using *P. afra* closely track the parent-source at the individual level as this may be a factor that may strongly influence the results.

Commented [RLO3]: I found this a bit tricky to follow and suggest it could be written more succinctly.

Commented [RLO4]: I would move this to after the description of the treatments.

Commented [RLO5]: New paragraph as this is now results?

Commented [RLO6]: But very importantly. This may be a key result!

Commented [RLO7]: Condition or genetics?

Introduction

The persistence of arid and semiarid ecosystems in a degraded state is often maintained by complex interactions operating at various spatial and temporal scales (D'Odorico et al., 2013; Evans and Geerken, 2004). Restoration of these ecosystems may thus require targeting key processes, such as erosion, herbivory, pathogens, and drought (James et al., 2013). A lack of reliable rainfall, for example, can restrict the regeneration of degraded arid and semiarid ecosystems, resulting in low rates of plant establishment (Valliere et al., 2019; Haase and Davis, 2017). Extended periods of low soil moisture may contribute to the poor survival of

Commented [RLO8]: Likely contributes

Commented [RLO9R8]: replace with 'and'

Portulacaria afra L. Jacq. cuttings planted in succulent thicket restoration initiatives, especially since the standard protocol is to plant unrooted cuttings—a long period of low soil moisture may retard or prevent rooting.

Succulent thicket represents the arid and semiarid components of the Albany Subtropical Thicket biome (termed "arid" and "valley" thicket in Vlok et al., 2003), which is endemic to South Africa. Although this vegetation occurs in a region that experiences aseasonal rainfall, with peaks in spring and autumn, it is still subject to frequent and often prolonged droughts that can extend across multiple years, including consecutive months with no rainfall (Mahlalela et al., 2020; Palmer et al., 2020; Vlok et al., 2003). Despite this, succulent thicket has been described as an evergreen dwarf forest (Midgley et al., 1997), characterised by a low canopy of trees and shrubs — usually with a canopy cover >70% — and an understory rich in succulents and geophytes (Vlok et al., 2003). A common and often distinguishing characteristic of succulent thicket is the abundance of the leaf and stem succulent tree, *P. afra,* which is frequently the most dominant species in terms of canopy cover and biomass (Guralnick and Gladsky, 2017; Vlok et al., 2003; Penzhorn et al., 1974). This species plays a key role in landscape-level facilitation, modifying local environmental conditions through shading effects (Wilman et al., 2014; Sigwela et al., 2009), intercepting rainfall (Cowling and Mills, 2011), and improving water infiltration by accumulating soil organic matter (van Luijk et al., 2013; Lechmere-Oertel et al., 2008). This facilitation is purported to enable the persistence of a closed-canopy scrub forest-like vegetation in arid areas (Lechmere-Oertel et al., 2005b).

Commented [RLO10]: Is this qualifier necessary in this audience?

Commented [RLO11]: Weakly seasonal

Commented [RLO12]: Simplify to 'multiyear droughts'

Livestock production has caused widespread and extreme degradation of succulent thicket vegetation, which exhibits limited evidence of natural regeneration (Sigwela et al., 2009; Lechmere-Oertel et al., 2005b). This degradation is ~~commonly~~ characterised by the complete loss of *P. afra* and the associated transition into a savanna-like habitat with limited ecological functioning (Lechmere-Oertel et al., 2005b, 2005a, 2008). Succulent thicket restoration, therefore, focuses predominantly on the reintroduction of *P. afra* as both a pioneer and ecosystem-engineering species.

The ability of *P. afra* to regenerate clonally is well documented (Oakes, 1973; MacOwens, 1897) and may reflect a co-evolution with elephant browsing (Stuart-Hill, 1992). This trait has been exploited in succulent thicket restoration programs as unrooted *P. afra* cuttings have established after being planted across a wide range of degraded thicket sites (van der Vyver et al., 2021a; Mills and Robson, 2017; Mills et al., 2015). The simplicity of *P. afra* reintroduction and potential for restored habitats to act as carbon sinks (van der Vyver and Cowling, 2019; Mills and Cowling, 2006, 2014) prompted experimental investigations into the feasibility of succulent thicket restoration at a biome scale (Mills et al., 2015; termed the “Thicket Wide Plot (TWP)” experiment).~~:~~ ~~an experiment of~~ 330 restoration plots (~~termed the “Thicket Wide Plot (TWP)” experiment~~) were established across the natural range of succulent thicket between 2008 and 2009; each plot ~~consisted~~ comprisiing~~of~~ a 50×50 m herbivore exclosure within which various unrooted planting treatments were tested (described in van der Vyver et al., 2021a). However, the survival within these plots ranged from zero to nearly 100% (van der Vyver et al., 2021b~~);~~ ~~t~~The factors responsible for the low survival ~~have been attributed~~include ~~to~~ frost, herbivory, and planting outside of the target habitat (Duker 2021; van der Vyver et al., 2021b), ~~however~~although~~,~~ the influence of weather conditions prior to and post planting could not be explored due to a lack of data.

As mentioned above, succulent thicket occurs within an aseasonal rainfall zone, receiving sporadic rainfall predominantly in autumn and spring, with an annual mean ranging from 100 to 450 mm (Vlok et al., 2003; Everard, 1987) and prolonged droughts are common with variable local rainfall patterns. Thus, the soil moisture necessary to stimulate and support root growth may be absent for long periods. The reasons for failed establishment (i.e., lack of rooting) remain unknown. Here we hypothesise that soil moisture in the first month after planting may affect the initiation of rooting and thus root growth of this succulent species, potentially providing insights into the variable survival reported in field plantings (van der Vyver, 2021b; Mills and Robson, 2017).

Commented [RLO13]: Perhaps integrate this section with the previous climate description to save on words

Commented [RLO14]: … periods that exceed the survival period of the cutting.

Materials & Methods

Harvesting

~~Samples~~ Branchlets were harvested on 27 July 2020 from a~~n urban site~~road verge within the city of Gqeberha, Eastern Cape, South Africa, where *P. afra* had been ~~used~~ planted approximately xx years ago to stabilise a ~~road verge — i.e. used for~~ slope.~~ rehabilitation~~; this experiment was conducted during the 2020 COVID-19 pandemic, where movement was highly restricted and thus material available for sampling was geographically limited. 84 branchlets were harvested ~~Material for the experiment was harvested~~ from seven randomly selected individual plants (hereafter "parent-plants").~~ From each parent-plant, a total of 84 branchlets were harvested~~; T~~t~~welve replicate branchlets per parent-plant were used in each of the seven watering treatments (described below). The stem length and basal diameter were recorded for each branchlette prior to planting; the branchlettes were smaller than the cuttings used in the standard planting protocol or the TWP experiment (also termed “truncheons” in van der Vyver et

Commented [RLO15]: Suggest this is unnecessary and implies that the approach was weakened by this.

Commented [RLO16]: Is this a term? If so, then use consistently throughout al., 2021a,b), with a stem width (mean ± sd) of 5.60±0.87 mm and a cutting length of 222±26 mm. Smaller branches were used (hence branchlette) to accommodate the limited space available in seedling trays and the glasshouse area.

~~All plants were harvested for the experiment on 27 July 2020.~~

Experimental layout

A total of twelve seedling trays, with planting cavities of 90 ml, were used for the experiment. All planting cavities were filled with ~~oven-dried~~ clay-rich soil obtained from an area supporting thicket. This soil was standardised by drying it in an oven at 60 °C until it reached a constant mass, then sieving it through a one-millimetre mesh to homogenise soil particle size.

All planting was conducted on 28 July 2020. Each tray included seven treatment rows, with one branchlette per parent-plant represented in each treatment. Each row was separated by an empty cavity to reduce shading effects and potential water overflow between adjacent treatments; also, all trays were raised to avoid water entering from the bottom of a cavity (see Figure S1 for more details). Treatment order was randomised across each tray, and parent-plant order was randomised in each treatment by row. All trays were placed in a ~~well lit~~well-lit portion of a glass house and randomly repositioned and rotated ~~on a~~ weekly ~~basis~~. Maximum and minimum temperature, and relative humidity were monitored in the glass house for the duration of the experiment. In brief, across the experiment period the mean±sd of the maximum temperature was 28.1±4.4℃, minimum temperature was 18.4±1.7℃, and relative humidity 53.9±10.2% (see Figure S2 for further details).

Commented [RLO17]: These 'results' perhaps should not be reported here, but rather in the figure S2

Watering treatments

The watering treatments represent a time staggered initial watering after planting. This included watering on the day of planting (D01), 4 days (D04), 7 days (D07), 14 days (D14), 21 days (D21), and 28 days (D28) after planting, and a control treatment (C) with no watering for the full duration of the experiment. After 32 days from the start of the experiment, all treatments were watered twice weekly for an additional two weeks. All watering events involved saturating the soil.

Data collection and analysis

After 42 days from initial planting ~~(8 September 2020)~~, all branchlettes were carefully removed from the soil and ~~rooting was evaluated (~~the percentage of cuttings per parent plant that rooted ~~within each~~per treatment was calculated). The roots ~~biomass~~ was removed from each

Commented [RLO18]: Why change the term to 'cuttings' here?

branchlette, and the soil was searched to ensure that all roots and root fragments were accounted for. Each branchlette's roots were rinsed to remove any excess soil, ~~and subsequently~~ dried at 60°C until constant weight, and weighed~~. Dry root mass was measured in grams to the third decimal place~~.

All analyses described below were performed in R v 4.2.1 (R Core Team, 2022). Differences in rooting success (percentage rooted cuttings within each plant across treatments) was evaluated via a nonparametric Kruskal-Wallace test (Hollander and Wolfe, 1973). The dry root mass produced during the experimental period was evaluated and compared using three approaches. Firstly, using a nested ANOVA approach ("RootMass~Treatment/Plant"; Chambers et al., 1992) coupled with a post-hoc Tukey test (Yandell, 1997). Secondly, Kruskal-Wallace tests were performed on the dry root mass across all treatments, and then also separating the data into individual parent plants across all treatments; post-hoc analyses were conducted on sets that returned <0.05 using the 'pgirmess' package (v 1.7.0; Giradoux, 2021). The unwatered control treatment was not included in the Kruskal-Wallace tests or nested ANOVA described above as no rooting was observed in this treatment. Finally, a more sophisticated approach using a linear mixed-effects modelling (LMM) approach was included; using the 'nlme' package (v 3.1-153; Pinheiro et al., 2018). To account for possible effects of stem width on root formation, as the larger area along the stem circumference of thicker stems may produce more roots, root mass was divided by stem circumference. The full fixed effects model was therefore represented as the standardised ($\bar{x} = 0$; $\sigma = 1$) root mass divided by stem circumference as a function of treatment, or *RootMass*/*StemCirc* ~ *Treatment*. The optimal random structure was determined by comparing the residual fit of separate full models (Zuur et al., 2009, 2010) that incorporated different combinations of either random intercepts per plant individual (random = ~1|*Plant*) from which the branchlette were taken or varying residual identity structure per plant (varIdent(form = ~1|*Plant*)). These were compared under restricted maximum likelihood estimated (REML) using the log-likehood ratio test and AIC scores (Zuur et al., 2009). The optimal residual variance structure included *Plant* individual as both a random intercept and identity structure. This model was then refitted under maximum likelihood estimation to test if the removal of the fixed effect (*Treatment*) substantially reduced the information criterion (model fit) score, which it did not ($\Delta AIC \leq 2$). The same model was then refitted under REML estimation and the 'summary' statistics of the fixed effects per *Treatment* compared using the t-statistic. The marginal and conditional R2 value for the most parsimonious model was calculated using the 'MuMIn' package (v. 1.47.1 Nakagawa and Schielzeth, 2013; Barton, 2022). All model assumptions in

Commented [RLO19]: I cannot comment on the appropriateness of the stats as I do not understand the various tests well enough.

Commented [RLO20]: Would this not have been better dealt with by treating stem diameter as a co-variable in the analysis, which would tell you if it is an important explanatory factor.

terms of homogeneity and normality of residuals were tested and met. The significance of statistical tests are reported according the terminology proposed by Muff et al. (2022).

Results and Discussion

Rooting window hypothesis

The timing of initial watering was found to have a moderate, significant effect on the proportion of *P. afra* cuttings that established roots (Figure 1; H5=11.352, p = 0.045). The total exclusion of watering (control) inhibited root initiation in all but one of 84 cuttings; this one cutting exhibited root tips and was considered not to have established roots. Early watering (within the first 4 days) resulted in the highest percentage of rooting across all parent-plants (Figure 1). However, there was only weak evidence for differences in dry root mass amongst treatments by the end of the experiment (F5,36 = 2.109, p = 0.063; H5=9.719, p=0.084; LMM: F5,489 = 1.832, p = 0.105), with some indication that root growth was maximised when watering began two to three weeks after planting (D14 and D21 in Figure 2, and higher coefficients in the LMM, although not significant: Table 1). The interaction terms with parent-plants is discussed separately in the section below entitled “Parent-plant effects”. These preliminary findings may suggest that while early onset of watering increased the success of root initiation, delayed watering might increase the relative accumulation of root mass in branchlettes that do set root. However, those plants with delayed watering also had a shorter period until the final four watering events that were applied to all treatments in the last two weeks (except the control) — this may have boosted root growth.

Commented [RLO21]: My poor remembrance of stats is crippling me and I battle to comment on the validity and presentation of the results in Table 1. SOrry

Commented [RLO22]: I don't see the significance of this statement or understand the biological mechanism that would give this result.

Root generation from stem cuttings consists of different processes: regeneration of damaged tissue at the wounding site, redifferentiation of cells to perform new functions (shoots become roots), root tip formation, and the elongation of root tissue to produce functioning roots (Bidabadi and Jain, 2020; Cameron and Thomson, 1969). These processes require different physiological conditions, and the outcome of cellular differentiation is influenced by the relative proportions of endogenous growth hormones (auxins promoting rooting initiation and cytokinins promoting root elongation) that are upregulated at the wound site in response to external stimuli (Fehér, 2019). The timing of watering *P. afra* cuttings may affect the abundance of these growth regulating hormones.

Commented [RLO23]: Unregulated? What does upregulated mean?

~~The authors~~We were unable to find studies of growth regulating hormones in succulent species; however, water availability and rooting have been explored in woody C3 cuttings (reviewed in De Almeida et al., 2017; da Costa et al., 2013). In general, as cuttings are unable to take up moisture until they are rooted, water stress responses are initiated soon after the removal of cuttings from the parent-plant, with the wound site resulting in rapid moisture loss. Early watering of cuttings can facilitate the maintenance of a positive water balance, preventing desiccation and the upregulation of secondary metabolites, which can inhibit cell cycle progression (Wolters and Jürgens, 2009). Soaking of fresh *Populus* cuttings in water prior to planting was found to improve water potential and stimulate rooting compared to unsoaked cuttings (Puri and Thompson, 2003). Dried cuttings had lower water potentials and initiated roots more slowly (Puri and Thompson, 2003). Similarly, cuttings of juniper (*Juniperus horizontalis*), azalea (Rhododendron), and holly (*Ilex crenata*) all exhibited improved root formation when planted into moisture rich substrates (Rein et al., 1991). Submerging apple tree (*Malus domestica* Borkh.) cuttings in water or agarose reduced the oxidation rate of endogenous auxins, resulting in improved rooting (de Klerk et al., 1999); however, high auxin concentrations have been found to reduce cell elongation and proliferation, slowing the growth of newly formed roots (de Klerk et al., 1999; Cameron and Thomson, 1969). The interaction of endogenous growth hormones that regulate root initiation and growth may explain the abundance of slower-growing roots in early watered *P. afra* cuttings and the rapid growth of few roots in later watered cuttings (D21 and D28, Figure 2; Table 1). However, further research is required to better understand the physiology of *P. afra* rooting and the effect of dry periods after rooting has been initiated.

Commented [RLO24]: This section is very speculative and the reality is there is no way to know if there is any effect or pattern at all to be explained due to the non-significance of the results. The results seem to be more driven by parent than any watering effect. I suggest the section could be reduced to a few sentences describing a hormone hypothesis that can be tested in future, and rather leave out the many examples of woody c3 plant responses.

Parent-plant effects

An unexpected observation in the data is that the source of the cuttings, i.e., the parent-plant, had a significant effect on the fraction of rooted cuttings and dry root mass (e.g., P3, P4 & P5, Figures 1 & 2; Table 2), providing strong evidence (F36,460 = 5.026, p <0.001) for a parent-plant interaction effect on root growth. This is demonstrated whereby including individual *Plant* identity in the LMM model as a random effect significantly improved its parsimony compared to the null model (df = 7, LR = 122.99, p < 0.001) and also that the model R2 fit when accounting only for the fixed effect (*Treatment*) was low (0.011) but substantially improved when the individual effect of *Plant* was included (0.219) (Table 1). The inclusion of both a random intercept and a residual variance structure per *Plant* identity within the most parsimonious model (all LR tests p < 0.001) suggests that there is both a parent-plant specific difference in the overall dry root mass accumulated per branchlette and a difference in the ~~the~~ variability between branchlettes from a single parent-plant in terms of overall dry root mass accumulated (i.e., some plants were more consistent or more variable in the dry root mass accumulated per branchlette than others). As the experimental design did not intend to describe parent-plant effects on rooting, we did not measure any in-field attributes of the parent-plants at the time of harvesting. Therefore, the discussion of this is speculative, but important, as tracking parent source material has not been included in the design of any experiments exploring *P. afra* establishment thus far (including the TWP experiment). Thus, it may be a hidden but significant confounding factor in such experiments.

Commented [RLO25]: I think this is the most important result of this paper and should be strongly emphasised for future research.

Cellular redifferentiation and growth require living material with sufficient internal resource supply for cell division. The ~~rootability~~ rooting success of cuttings is potentially affected by tissue age (studied in *Diploknema butyracea*: Zargar and Kumar, 2018; *Dalbergia melanoxylon:* Amri et al., 2010; *Tectona grandis*: Husen and Pal, 2007; *Quercus spp.:* Chalupa, 1993) and orientation of the cuttings on the parent-plant (studied in *Dalbergia melanoxylon:* Amri et al., 2010; *Tectona grandis*: Husen and Pal, 2007b; *Dalbergia sissoo*: Husen, 2004). This effect on rooting is due to the influence these two factors have on the availability of internally stored resources such as endogenous growth hormones and carbohydrates (soluble sugars and starch). Stored carbohydrates provide the energy required for cell division and, thus, are required for root formation and growth (Husen and Pal, 2007a). Furthermore, growth hormones such as auxins play a role in the metabolism and mobilisation of carbohydrates (Ruedell et al., 2015; Husen, 2008). A lack of either of these resources can limit the rooting potential of cuttings (Amri et al., 2010). Older plant tissue tends to exhibit reduced carbohydrate content (Husen, 2008; Husen and Pal, 2007a), a loss of sensitivity to growth hormones, decreased endogenous growth hormone content, and an accumulation of growth inhibitory substances (Bidabadi and Jain, 2020; Zargar and Kumar, 2018; Ikeuchi et al., 2016; Amri et al., 2010; Husen and Pal, 2007a). Similarly, the source location of cuttings from their parent plant can influence carbohydrate content and sensitivity to growth hormones (Amri et al., 2010; Husen and Pal, 2007b; Husen, 2004; Zalesny et al., 2003). These studies show that cuttings taken from different positions (apical, basal, or mid stem) therefore exhibit different rooting potentials. The position in which to source cuttings, however, appears to be species-specific and was not consistent across the studies cited here. As tissue age and cutting location were not considered while harvesting *P. afra* cuttings, nor were the overall state of the plant or its recent history, these factors may have affected rooting at the parent-plant level. Each individuals' cuttings were sourced from a range of branches (3-5) that were harvested and later split into multiple cuttings of similar size for propagation. Therefore, the cuttings from an individual may have varied in relative position from the parent-plant and age. All the plants were harvested from the same macro-environmental conditions, i.e., from the same slope and within 20 m of each other. Currently, the effect of the parent source material on the cutting establishment and survival has not been tested in *P. afra* restoration, but this should be explored at the intra- and inter-plant level to inform future harvesting ~~practises~~practices for thicket restoration.

Implications for thicket restoration

Succulent thicket restoration currently utilises unrooted *P. afra* cuttings that are harvested indiscriminately and planted with limited regard for the wetter spring and autumn months (Mills et al., 2015). This approach has resulted in variable success, with survival ranging between zero % and almost 100% (mean survival estimated to be 28%) in large scale plantings (Mills and Robson, 2017). The low survival reported has since been attributed to uncontrolled herbivory and planting into the incorrect target habitat (van der Vyver et al., 2021b), with a large number of experimental plots established in adjacent frost-prone shrublands (Duker et al., 2020) that cannot support the frost sensitive *Portulacaria afra* (Duker et al., 2015).

The potential effects of rainfall post planting and *P. afra* source material have not previously been considered in the evaluation of factors influencing cutting survival under field conditions. While watering at planting was included in the TWP experiment, it was not found to have a significant impact on *P. afra* cutting survival (van der Vyver et al., 2021a). In contrast, our results suggest that early access to water does affect rooting in *P. afra* cuttings; however, a waterless period beyond 28 days needs to be tested. The lack of a watering effect reported by van der Vyver et al. (2021a) may be a consequence of rainfall soon after planting, nullifying the treatment effect, or long periods (multiple months) of no rainfall post-planting, which are common in succulent thicket habitats (Mahlalela et al., 2020; Palmer et al., 2020; Vlok et al., 2003). Furthermore, if material is not randomised, the use of cuttings harvested from poor parent material (i.e., harvesting from a nearby, old, or physiologically stressed *P. afra* population) may impact the survival of entire experimental plots or treatments within plots. Thus, further complicating the interpretation of the TWP results.

Commented [RLO26]: But, your Fig 2 data suggests the overall treatment effect has very low statistical significance, and you suggest above (lines 200-212) that delayed watering may be beneficial for root production. This seems a contradiction.

Further work is required to better describe the processes underpinning the parent-plant effect and the water requirements for initiating rooting in *P. afra* as this information may inform best ~~practises~~practices for future restoration initiatives. However, the results presented here suggest that soil moisture is required relatively soon after planting to facilitate root formation. Planting efforts should, therefore, be timed to best take advantage of the rainfall patterns specific to the restoration site in question, and seasonal planting of cuttings sourced from the best available

Commented [RLO27]: As above

parent material during the wetter autumn and spring months may be advised; however, there can be substantial inter-annual variation in these bimodal rainfall peaks.

Conclusions

The restoration of succulent thicket, a dwarf forest vegetation endemic to South Africa, is dependent on better understanding the regeneration dynamics of the primary target plant species, *P. afra*. This study provides evidence that the timing of water exposure post planting of stem cuttings may impact rooting. Unexpectedly, strong evidence for a parent-plant effect on rooting was also detected. These results highlight the complexity of interpreting the drivers of *P. afra* survival under field conditions. Further work is required to better understand the parent-plant effect documented here and to inform restoration practices.

Commented [RLO28]: "has little to no significant impact on rooting success"

Acknowledgements

References

Amri, E., Lyaruu, H.V.M., Nyomora, A.S., Kanyeka, Z.L., 2010. Vegetative propagation of African Blackwood (*Dalbergia melanoxylon* Guill. & Perr.): effects of age of donor plant, IBA treatment and cutting position on rooting ability of stem cuttings. New Forests 39: 183–194. https://doi.org/10.1007/s11056-009-9163-6

Barton, K., 2022. MuMIn: multi-model inference. R package version 1. 47. 1. *http://r-forge. r-project. org/projects/mumin/*

Bidabadi, S.S., Jain, S.M., 2020. Cellular, molecular, and physiological aspects of in vitro plant regeneration. Plants 9: 702. https://doi.org/10.3390/plants9060702

Cameron, R.J., Thomson, G.V., 1969. The vegetative propagation of *Pinus radiata*: Root initiation in cuttings. Botanical Gazette 130: 242–251. https://doi.org/10.1086/336498

Chalupa, V., 1993. Vegetative propagation of oak (*Quercus robur* and *Q. petraea*) by cutting and tissue culture. Annals of Forest Science 50: 295s–307s. https://doi.org/10.1051/forest:19930730

Chambers J.M., Freeny A.E., Heiberger R.M., 1992. Analysis of variance; designed experiments. In Statistical models in Science. Routledge, Oxfordshire, England, pp. 145-193.

Cowling, R.M., Mills, A.J., 2011. A preliminary assessment of rain throughfall beneath *Portulacaria afra* canopy in subtropical thicket and its implications for soil carbon stocks. South African Journal of Botany 77: 236–240. https://doi.org/10.1016/j.sajb.2010.06.004

de Klerk, G.-J., van der Krieken, W., de Jong, J.C., 1999. Review the formation of adventitious roots: New concepts, new possibilities. In Vitro Cellular & Developmental Biology - Plant 35: 189–199. https://doi.org/10.1007/s11627-999-0076-z

D'Odorico, P., Bhattachan, A., Davis, K.F., Ravi, S., Runyan, C.W., 2013. Global desertification: drivers and feedbacks. Advances in Water Resources 51: 326–344.

Duker, R., Cowling, R.M., du Preez, D.R., Potts, A.J., 2015. Frost, *Portulacaria afra* Jacq., and the boundary between the Albany Subtropical Thicket and Nama-Karoo biomes. South African Journal of Botany 101: 112–119. https://doi.org/10.1016/j.sajb.2015.05.004

Duker, R., Cowling, R.M., van der Vyver, M.L., Potts, A.J., 2020. Site selection for subtropical thicket restoration: mapping cold-air pooling in the South African sub-escarpment lowlands. PeerJ 8: e8980. https://doi.org/10.7717/peerj.8980

Evans, J., Geerken, R., 2004. Discrimination between climate and human-induced dryland degradation. Journal of Arid Environments 57: 535–554. https://doi.org/10.1016/S0140-1963(03)00121-6

Everard, D.A., 1987. A classification of the subtropical transitional thicket in the Eastern Cape, based on syntaxonomic and structural attributes. South African Journal of Botany 53: 329–340. https://doi.org/10.1016/S0254-6299(16)31393-X

Fehér, A., 2019. Callus, dedifferentiation, totipotency, somatic embryogenesis: What these terms mean in the era of molecular plant biology? Frontiers in Plant Science 10: 536. https://doi.org/10.3389/fpls.2019.00536

Giraudoux, P., 2021. pgirmess: Spatial Analysis and Data Mining for Field
Ecologists. R package version 1.7.0. https://CRAN.R-project.org/package=pgirmess

Guralnick, L.J., Gladsky, K., 2017. Crassulacean acid metabolism as a continuous trait: variability in the contribution of Crassulacean acid metabolism (CAM) in populations of *Portulacaria afra*. Heliyon 3: e00293. https://doi.org/10.1016/j.heliyon.2017.e00293

Haase, D., Davis, A., 2017. Developing and supporting quality nursery facilities and staff are necessary to meet global forest and landscape restoration needs. REFORESTA 69–93. https://doi.org/10.21750/REFOR.4.06.45

Hollander, M., Wolfe, D.A., 1973. Nonparametric Statistical Methods. John Wiley & Sons, New York.

Husen, A., 2008. Clonal propagation of *Dalbergia sissoo* Roxb. and associated metabolic changes during adventitious root primordium development. New Forests 36: 13–27. https://doi.org/10.1007/s11056-007-9079-y

Husen, A., 2004. Clonal Propagation of *Dalbergia sissoo* Roxb. by softwood nodal cuttings: Effects of genotypes, application of IBA and position of cuttings on shoots. Silvae Genetica 53: 50–55. https://doi.org/10.1515/sg-2004-0009

Husen, A., Pal, M., 2007a. Metabolic changes during adventitious root primordium development in *Tectona grandis* Linn. f. (teak) cuttings as affected by age of donor plants and auxin (IBA and NAA) treatment. New Forests 33: 309–323. https://doi.org/10.1007/s11056-006-9030-7

Husen, A., Pal, M., 2007b. Effect of branch position and auxin treatment on clonal propagation of *Tectona grandis* Linn. f. New Forests 34: 223–233. https://doi.org/10.1007/s11056-007-9050-y

Ikeuchi, M., Ogawa, Y., Iwase, A., Sugimoto, K., 2016. Plant regeneration: cellular origins and molecular mechanisms. Development 143, 1442–1451. https://doi.org/10.1242/dev.134668

James, J.J., Sheley, R.L., Erickson, T., Rollins, K.S., Taylor, M.H., Dixon, K.W., 2013. A systems approach to restoring degraded drylands. Journal of Applied Ecology 50: 730–739. https://doi.org/10.1111/1365-2664.12090

Lechmere-Oertel, R.G., Cowling, R.M., Kerley, G.I.H., 2005a. Landscape dysfunction and reduced spatial heterogeneity in soil resources and fertility in semi-arid succulent thicket, South Africa. Austral Ecology 30: 615–624. https://doi.org/10.1111/j.1442-9993.2005.01495.x

Lechmere-Oertel, R.G., Kerley, G.I.H., Cowling, R.M., 2005b. Patterns and implications of transformation in semi-arid succulent thicket, South Africa. Journal of Arid Environments 62: 459–474. https://doi.org/10.1016/j.jaridenv.2004.11.016

Lechmere-Oertel, R.G., Kerley, G.I.H., Mills, A.J., Cowling, R.M., 2008. Litter dynamics across browsing-induced fenceline contrasts in succulent thicket, South Africa. South African Journal of Botany 74: 651–659. https://doi.org/10.1016/j.sajb.2008.04.002

MacOwens, P., 1897. Part 1 - Stock farming, in: Agricultural Miscellanea: Extracts from Volumes 1 to 5 of the “Agricultural Journal”. W. A. Richards and Sons, Cape Town, South Africa, pp. 193–194.

Mahlalela, P.T., Blamey, R.C., Hart, N.C.G., Reason, C.J.C., 2020. Drought in the Eastern Cape region of South Africa and trends in rainfall characteristics. Climate Dynamics 55: 2743–2759. https://doi.org/10.1007/s00382-020-05413-0

Midgley, J.J., Cowling, R.M., Seydack, A.H.W., Van Wyk, G.F., 1997. Forest, in: Vegetation of Southern Africa. Cambridge University Press, Cambridge, pp. 278–295.

Mills, A., Vyver, M., Gordon, I., Patwardhan, A., Marais, C., Blignaut, J., Sigwela, A., Kgope, B., 2015. Prescribing innovation within a large-scale restoration programme in degraded Subtropical Thicket in South Africa. Forests 6, 4328–4348. https://doi.org/10.3390/f6114328

Mills, A.J., Cowling, R.M., 2014. How fast can carbon be sequestered when restoring degraded subtropical thicket?: Carbon sequestration in restored subtropical thicket. Restoration Ecology 22: 571–573. https://doi.org/10.1111/rec.12117

Mills, A.J., Cowling, R.M., 2006. Rate of carbon sequestration at two thicket restoration sites in the Eastern Cape, South Africa. Restoration Ecology 14: 38–49. https://doi.org/10.1111/j.1526-100X.2006.00103.x

Mills, A.J., Robson, A., 2017. Survivorship of spekboom (*Portulacaria afra*) planted within the Subtropical Thicket Restoration Programme. South African Journal of Science 113: https://doi.org/10.17159/sajs.2017/a0196

Muff, S., Nilsen, E.B., O'Hara, R.B. and Nater, C.R., 2022. Rewriting results sections in the language of evidence. Trends in Ecology & Evolution 37: 203–210. https://doi.org/10.1016/j.tree.2021.10.009

Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4: 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

Oakes, A.J., 1973. *Portulacaria afra* Jacq. — A potential browse plant. Economic Botany 27: 413–416. https://doi.org/10.1007/BF02860694

Palmer, A.R., Ezenne, G.I., Choruma, D.J., Gwate, O., Mantel, S.K., Tanner, J.L., 2020. A comparison of three models used to determine water fluxes over the Albany Thicket, Eastern Cape, South Africa. Agricultural and Forest Meteorology 288–289: 107984. https://doi.org/10.1016/j.agrformet.2020.107984

Penzhorn, B.L., Robbertse, P.J., Olivier, M.C., 1974. The influence of the African Elephant on the vegetation of the Addo Elephant National Park. Koedoe 17: 137–158. https://doi.org/10.4102/koedoe.v17i1.906

Pinheiro, J, Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2021. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-153. https://CRAN.R-project.org/package=nlme

Puri, S., Thompson, F. B., 2003. Relationship of water to adventitious rooting in stem cuttings of *Populus* species. Agroforestry Systems 58: 1–9. https://doi.org/10.1023/A:1025494221846

R Core Team, 2022. RStudio: Integrated Development Environment for R.

Rein, W. H., Wright, R. D., Seiler, J. R., 1991. Propagation medium moisture level influences adventitious rooting of woody stem cuttings. Journal of the American Society for Horticultural Science 116: 632–636. https://doi.org/10.21273/JASHS.116.4.632

Ruedell, C.M., de Almeida, M.R., Fett-Neto, A.G., 2015. Concerted transcription of auxin and carbohydrate homeostasis-related genes underlies improved adventitious rooting of microcuttings derived from far-red treated *Eucalyptus globulus* Labill mother plants. Plant Physiology and Biochemistry 97: 11–19. https://doi.org/10.1016/j.plaphy.2015.09.005

Sigwela, A.M., Kerley, G.I.H., Mills, A.J., Cowling, R.M., 2009. The impact of browsing-induced degradation on the reproduction of subtropical thicket canopy shrubs and trees. South African Journal of Botany 75: 262–267. https://doi.org/10.1016/j.sajb.2008.12.001

Stuart-Hill, G.C., 1992. Effects of elephants and goats on the kaffrarian succulent thicket of the Eastern Cape, South Africa. Journal of Applied Ecology 29: 699-710. https://doi.org/10.2307/2404479

Valliere, J.M., Zhang, J., Sharifi, M.R., Rundel, P.W., 2019. Can we condition native plants to increase drought tolerance and improve restoration success? Ecological Applications 29: e01863 https://doi.org/10.1002/eap.1863

van der Vyver, M.L., Cowling, R.M., 2019. Aboveground biomass and carbon pool estimates of *Portulacaria afra* (spekboom)-rich subtropical thicket with species-specific allometric models. Forest Ecology and Management 448: 11–21. https://doi.org/10.1016/j.foreco.2019.05.048

van der Vyver, M.L., Mills, A.J., Cowling, R.M., 2021a. A biome-wide experiment to assess the effects of propagule size and treatment on the survival of *Portulacaria afra* (spekboom) truncheons planted to restore degraded subtropical thicket of South Africa. PLOS ONE 16, e0250256. https://doi.org/10.1371/journal.pone.0250256

van der Vyver, M.L., Mills, A.J., Difford, M., Cowling, R.M., 2021b. Herbivory and misidentification of target habitat constrain region-wide restoration success of spekboom (*Portulacaria afra*) in South African subtropical succulent thicket. PeerJ 9, e11944. https://doi.org/10.7717/peerj.11944

van Luijk, G., Cowling, R.M., Riksen, M.J.P.M., Glenday, J., 2013. Hydrological implications of desertification: Degradation of South African semi-arid subtropical thicket. Journal of Arid Environments 91: 14–21. https://doi.org/10.1016/j.jaridenv.2012.10.022

Vlok, J.H.J., Euston-Brown, D.I.W., Cowling, R.M., 2003. Acocks’ Valley Bushveld 50 years on: new perspectives on the delimitation, characterisation and origin of subtropical thicket vegetation. South African Journal of Botany 69: 27–51. https://doi.org/10.1016/S0254-6299(15)30358-6

Wilman, V., Campbell, E.E., Potts, A.J., Cowling, R.M., 2014. A mismatch between germination requirements and environmental conditions: Niche conservatism in xeric subtropical thicket canopy species? African Journal of Botany 92: 1–6. https://doi.org/10.1016/j.sajb.2013.12.007

Wolters, H., Jürgens, G., 2009. Survival of the flexible: hormonal growth control and adaptation in plant development. Nature Reviews Genetics volume 10: 305–317. https://doi.org/10.1038/nrg2558

Yandell, B.S., 1997. Practical Data Analysis for Designed Experiments, 1st ed. Routledge, Oxfordshire, England. https://doi.org/10.1201/9780203742563

Zalesny, R.S., Hall, R.B., Bauer, E.O., Riemenschneider, D.E., 2003. Shoot position affects root initiation and growth of dormant unrooted cuttings of *Populus*. Silvae Genetica 52: 273–279.

Zargar, A.R., Kumar, D., 2018. Effect of maturity stage of donor plant on propagation of *Diploknema butyracea* through branch cuttings. World Journal of Agricultural Research 6: 15-19. DOI: 10.12691/wjar-6-1-4

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecology and Evolution 1: 3–14.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., others, 2009. Mixed effects models and extensions in ecology with R. Springer.

REVIEWER’S GENERAL COMMENTS

The study is simple and well-designed in principle, although the short duration of the experiment does hamper the key conclusion of the ‘rooting window’.

The experimental design is good and allows what seems to be the appropriate stats (apologies for my lack of comment on the stats).

The findings are valid in themselves, although I tend to think the key result is the lack of significant rooting pattern that comes from the treatment effect. It would appear to me that the statement that water is NOT needed for rooting success (within the time frame of this experiment) gives hope to the massive restoration projects that cannot provide water at planting or thereafter. In this regard, I suggest some of the key statements in the discussion and conclusion could be re-articulated to reflect this, as opposed to saying that early watering does promote rooting.

The figures and tables are well laid out and understandable.

I found the discussion section that tries to explain (a non-existent?) pattern of rooting response to watering at a hormonal level a bit too speculative. I think there may be truth in the speculation, but the authors quote many unrelated studies that are not easily comparable, and no conclusion can actually be drawn from the section. I would trim this a bit.

The incidental result regarding the parent material is such an important result as it COULD offer the key to understanding the variable success of previous field plantings. I would more strongly emphasise this aspect.