Planting orientation of *Portulacaria afra* cuttings for Thicket restoration: vertical versus horizontal

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Aim. The re-establishment of Portulacaria afra in the landscape-scale Subtropical Thicket Restoration Programme has exclusively used vertically-orientated truncheons (i.e. large cuttings with the main stem planted 10-20 cm into the ground). Despite the planting of millions of truncheons, the rates of survival, growth and restoration are low. This may be driven by browsing pressure and/or drought conditions during the truncheon establishment phase. Here we conduct a common garden experiment to explore the establishment of horizontal versus vertically orientated truncheons. Horizontal truncheons have their main stem buried in the soil and only a few side branches exposed above ground — these truncheons may experience reduced water stress. Here we compared the levels of water stress during the establishment phase of truncheons with different orientation.

Location. Eastern Cape, South Africa.

Methods. Our experiment involved three planting treatments for truncheons: vertical orientation, horizontal orientation, and horizontal orientation with exposed side branches clipped. Truncheons were grown for two months and plants were well-watered. On two occasions during the experiment, photosynthetic efficiency was measured on all plants to ascertain levels of plant stress. After the experiment, the root, stem and leaf dry mass were recorded for each replicate, as well as leaf moisture.

Results. The root mass proportion (of the total plant) was not significantly different among treatments. Despite this, leaf-level photosynthetic efficiency was recorded as significantly lower in vertical truncheons versus horizontal truncheons.

Main conclusions. Smaller horizontally-orientated truncheons do not grow roots at a faster rate (relative to their total size) than the larger vertically-orientated truncheons that have more leaf material to support. Nonetheless, under well-watered conditions, the larger truncheons experienced stress evidenced by lowered leaf photosynthetic efficiency values. Thus, we suggest that horizontal buried truncheons may have a higher likelihood of survival under seasonal drought-stress conditions. It remains to be tested whether horizontally-orientated truncheons (with less above-ground biomass) experience lower rates of herbivory than the standard vertical cuttings.

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Abstract

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Keywords : spekboom restoration; planting orientation; photosynthetic efficiency; moisture content.

Introduction

Much of the Albany Subtropical Thicket vegetation of South Africa (hereafter referred to as "thicket") has experienced extensive degradation, spanning many decades, due to overstocking of livestock, primarily goats (Sigwela et al. 2009). Sustained heavy browsing by domestic livestock can transform the canopy from dense closed-canopy shrubland into an open landscape comprising remnant trees or thicket clumps within a field layer of ephemeral herbs (Stuart-Hill, 1992; Lechmere-Oertal et al. 2005; Sigwela et al. 2009). One of the abundant species in this vegetation is especially sensitive to overbrowsing by domestic livestock: the succulent tree *Portulacaria afra* (L.) Jacq. (locally termed "spekboom")(Stuart-Hill, 1992). After severe degradation, this species does not re-establish naturally even if livestock and game stocking densities are reduced (Lechmere-Oertel, 2008).

Portulacaria afra is considered an ecosystem engineer in the more arid forms of thicket as it increases soil moisture post-rainfall (van Luijk et al. 2013), and provides an environment for seedlings of subtropical tree lineages to establish (Wilman et al. 2014). It is also drought-tolerant and can shift from C3 photosynthesis to CAM or CAM-idling under low water conditions (Guralnik and Ting, 1987; Guralnick and Gladsky, 2017). Also, *P. afra* readily resprouts from stems or leaves, and plants establish from planted cuttings without irrigation or cultivation in a nursery — cuttings of *P. afra* can be simply established by harvesting and planting directly into the landscape for restoration (van der Vyver et al., 2012; Fig. 1A). This species is currently the most widely used for large-scale restoration of degraded thicket landscapes in South Africa (Mills et al., 2007; Mills et al., 2015) and other species are not considered economically viable (van der Vyver 2012). The planting of *P. afra* cuttings has been mainstreamed by the South African government in a combined environmental restoration and poverty alleviation program known as Subtropical Thicket Restoration Programme (Mills et al. 2015). As part of this program, for example, an approximate 21 million cuttings were planted over the period of 2004–2016 across the Addo Elephant National Park, Great Fish River Nature Reserve and the Baviaanskloof Nature Reserve (Mills and Robson, 2017). However, survival was very low with an overall average of 72% mortality (Mills and Robson, 2017). High mortality is a general problem, as is the lower-than-expected growth rates (Mills et al. 2015), and this has hampered the restoration initiative.

The standard restoration protocol involves planting large upright cuttings of *P. afra*, often referred to as spekboom "truncheons", with a basal diameter of ~25 mm (Mills et al. 2015; Fig. 1A). Due to the high mortality rate observed using vertical planting, we compared three planting treatments for truncheons: vertical orientation, horizontal orientation, and horizontal orientation with their exposed side branches clipped. We measured photosynthetic efficiency, root, stem and leaf dry mass, as well as leaf moisture for all replicates. We expected that the horizontal orientation would lead to faster root growth relative to the overall cutting size as the there would be relatively less photosynthetic organs to support (side branches were trimmed from these cuttings). As all treatments would be well-watered (relative to field conditions), we also expected that no treatment should exhibit signs of drought stress. From our results, we propose a new planting orientation for spekboom restoration, using smaller cutting sizes.

Materials and Methods

A common garden experiment was conducted to test the effect of planting orientation on rooting and leaf photosynthetic efficiency; the experiment was undertaken from the 6 March 2018 to 6 May 2018 (total: 61 days). We explored how cuttings planted horizontally compare with those planted using the standard vertical truncheon in terms of proportional root growth and evidence of drought stress (using leaf chlorophyll fluorescence as a proxy of water stress; Woo et al. 2008). Horizontally-orientated cuttings were planted with main stem buried and side branches exposed above ground (Fig. 1C&D versus Fig. 1B). We also included a second horizontal planting treatment of clipping the side branches at ~10 cm (Fig. 1C); herbivory is a major threat to spekboom cuttings, especially in the establishment phase were entire cuttings are easily removed and reducing the above-ground component would decrease the visibility of cuttings during establishment. Cuttings are planted without roots, thus ratio of root to shoots is unbalanced and this will affect overall growth rates (Iwasa and Roughgarden, 1984). Fifteen replicates were used per treatment set (i.e. vertical planting, horizontal planting, and horizontal planting with emergent branches clipped). Thus, the experiment consisted of a total of 45 plants.

The cuttings were harvested from a horticultural plantation of *P. afra* grown in Port Elizabeth. The plantation consisted of a dense stand of large (>1.5 m) healthy plants. Cuttings were planted on the day they were harvested. No cuttings showed any visible signs of stress in leaves or stems at the time of harvesting or planting.

Cuttings were planted individually in plastic pots (height: 30 cm; diameter: 20 cm). The soil mixture consisted of sifted topsoil from Enon conglomerate and shale-derived soils; *P. afra*, and thicket vegetation in general, commonly occurs on such soils and *P. afra* is not selective regarding soils (Mills et al. 2011). For the vertical orientation treatment, side branches were trimmed from the lower 15-20 cm of the main stem, and then the each cutting was planted to a depth of 15 cm in the pot; this follows the general recommendation of the Subtropical Thicket Restoration Programme protocol. In the case of horizontally-orientated cuttings, the main stem was trimmed to 10-15 cm and two side stems (on the same side) were kept while the rest were trimmed. The main stem was planted ~5 cm below the soil with the untrimmed side stem above ground (Fig. 1C and 1D). See Supplementary Figures S1 and S2 for further explanatory photographs. All plants were watered weekly and six rainfall events occurred during the experiment (five below 5 mm and one of 19 mm [Day 10]; a timeline of events is provided in Table S1).

To monitor plant stress during the experiment, we recorded leaf-level photosynthetic efficiency (F_V/F_m) values on two occasions (9 and 25 April; i.e. Days 34 and 50; Table S1). On each occasion, five F_V/F_m readings (each on a different leaf) were recorded per plant using Handy Plant Efficiency Analyser (PEA; Hansatech, Norfolk, United Kingdom); all measurements were conducted at least an hour after sunset. Photosynthetic efficiency ratios (F_V/F_m) provide an understanding of the state of photosystem II (PSII) by measuring the chlorophyll fluorescence (Murchie and Lawson, 2013). Chlorophyll fluorescence analysis is a non-invasive and easy measure that indicates tolerance of plants to environmental stress (Paknajeb et al., 2007; Murchie and Lawson, 2013).

After 60 days, plants were removed from the pots, and roots, stems and a sample of leaves were separated and dried; the buried part of the original stem was *not* included as part of the root mass. In addition, an approximate 8-16 g of leaf material was sampled from each plant to determine leaf moisture; some (n=9) plants had fewer leaves and less than 8 g was sampled. All material was dried at 60℃ for five days. Note that no new stem or leaf growth was observed in any cutting at the time of harvesting (new shoot growth is characterised by having a red stem).

All statistical tests were conducting in R version 3.5.1 (R Core Team, 2018). Parametric and nonparametric statistics (ANOVA and Kruskal-Wallis; *stats* library: *aov*() and *kruskal.test*(), respectively) were conducted on the root mass percentage, mean (per plant) F_v/F_m values and leaf moisture. The post-hoc parametric Tukey Honest Significant Differences test (*stats* library: *TukeyHSD*()) and nonparametric Dunn's test for multiple comparison using rank sums (*dunn.test* library, version *1.3.5*: *dunn.test*() function) were conducted to assess significant differences between treatments. Root mass percentage values were compared with those from unpublished data (L. Guralnick and A.J. Potts) of *P. afra* plants grown from seed; seedlings (n=48) were harvested between five and ten months after planting and weight variation ranged between 0.02 and 3.99 g (dry mass of total plant).

Results and Discussion

The cuttings in the vertical treatment produced significantly more roots than those in the horizontal treatment (Fig. 2A; Fig. S3); however, this was not unexpected as the vertical cuttings were far larger, with far greater stem mass, than those used in the two horizontal treatments. Nonetheless, and contrary to our expectations, when comparing the root mass percentage (of the total plant), there were no statistical differences amongst the three treatments (Fig. 2B). In addition, if we assume that the seedling root proportion reporting in Figure 2 is also representative of larger plants, then by the end of 60 days, all three treatments had rooting proportions far lower than seed-grown plants. This is not unexpected given the short duration of the experiment. However, this imbalance between rooting and the remainder of the cutting was evident in the leaf physiology. On the larger vertical cuttings, despite a generous weekly watering regime and this treatment having significantly higher overall root biomass, the leaves were showing visible signs of stress (e.g. shrinking and wrinkling). This was confirmed by measurements of photosynthetic efficiency as the vertically-orientated treatment had significantly lower F_v/F_m values than the horizontal treatment (Fig. 3). Only severe stress can cause such a reduction in F_v/F_m (Ritchie, 2006). In addition, the leaves of the vertical treatment had significantly lower moisture content than the horizontal treatment (Fig. S4). We suspect that this stress was caused by the proportionally greater amount of leaf material that the larger vertical treatment cuttings had to support (and few leaves were shed by any plant). Thus, our results suggest that the horizontal orientation and burying the primary stem may improve the drought-tolerance, survival, and establishment of *P. afra* cuttings in degraded landscapes.

There is little information in the literature to compare the effect of orientation on planting success in drought-adapted or succulent tree cuttings. This topic has been almost exclusively explored across a range of woody species that are associated with high soil moisture (e.g. *Bambusa vulgaris*: Bhol and Nayak, 2012; *Dalbergia sissoo*: Chatuvedi, 2001; *Salix schwerinii x Salix viminalis*: Lowthe-Thomas et al. 2010 and Edelfedt et al. 2015). In addition, there is no consistency amongst the results from these studies, as horizontal planting does not consistently outperform upright planting in terms of sprouting, rooting, survival or biomass.

Portulacaria afra is generally found in semi-arid landscapes and is drought-tolerant (Vlok et al., 2003). Its dominance and density in thicket has led to its recognition as a vegetation type, i.e. “spekboomveld” (Acocks, 1953). Spekboom abundance has also been included in the vegetation descriptions of thicket by Vlok et al. (2003), i.e. Spekboomveld (in arid thicket types), and Spekboom Thicket (in valley thicket types). These vegetation types are found in semi-arid regions where mean annual precipitation ranges from ~150 to 600 mm. Both Spekboomveld and Spekboom Thicket units have high proportions of moderately and severely degraded vegetation, generally >50% (Vlok and Euston-Brown, 2002; Lloyd et al. 2003), and are thus targets for restoration efforts. In addition to the generally arid environment, rainfall is highly variable and both short-term and long-term droughts are common climatic occurrences in the Eastern Cape landscape (Jury and Levey, 1993) and over these thicket types. Thus, despite the success of large cuttings after establishment (e.g. Mills & Cowling, 2006), our results suggest that large cuttings may experience an extended period of drought-stress due to the imbalance between leaf and root biomass. However, in the Thicket-wide plot experiment (a large-scale ecological restoration experiment with 300 plots situated across the Thicket biome of South Africa; see van der Vyver 2017 for more details), smaller cuttings (i.e. 10 mm basal stem diameter) had significantly lower survival than larger truncheons (i.e. 30 mm basal stem diameter) (van der Vyver 2017). This was likely driven by the lower water storage capacity of the smaller stem volumes in the smaller cuttings. Here, we suggest that the horizontal orientation, which includes trimming of side branches, reduces the leaf biomass that needs to be supported but, importantly, still provides the water holding capacity in the buried stem. Stem water storage is a crucial adaptation in arid environments with unpredictable rainfall (Holbrook, 1995), and *Portulacaria afra* has a high capacity to store water in its stems, with stem water content ~70% (versus <40% in woody trees; Fig. S5). Thus, we suggest that by reducing the demands required by the above-ground stems and leaves while maintaining the stem water storage capacity in the buried primary stem, there may be a significant increase in cutting survival in the field. A series of field experiments in different environments and climatic sequences is required to test this.

Another possible benefit of having the primary stem of the cutting buried with limited above-ground branches is the reduced influence of herbivory. Van der Vyver (2017) identified herbivory as a significant predictor of mortality within the Thicket-wide plot experiment, and suggested that herbivory likely drives the unexplained low survival observed in monitoring of large-scale plantings of *P. afra* (e.g. Mills and Robson, 2017). Testing whether horizontally-orientated truncheons (with less above-ground biomass) experience lower rates of herbivory than the standard vertical cuttings in the field requires urgent attention.

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Figures

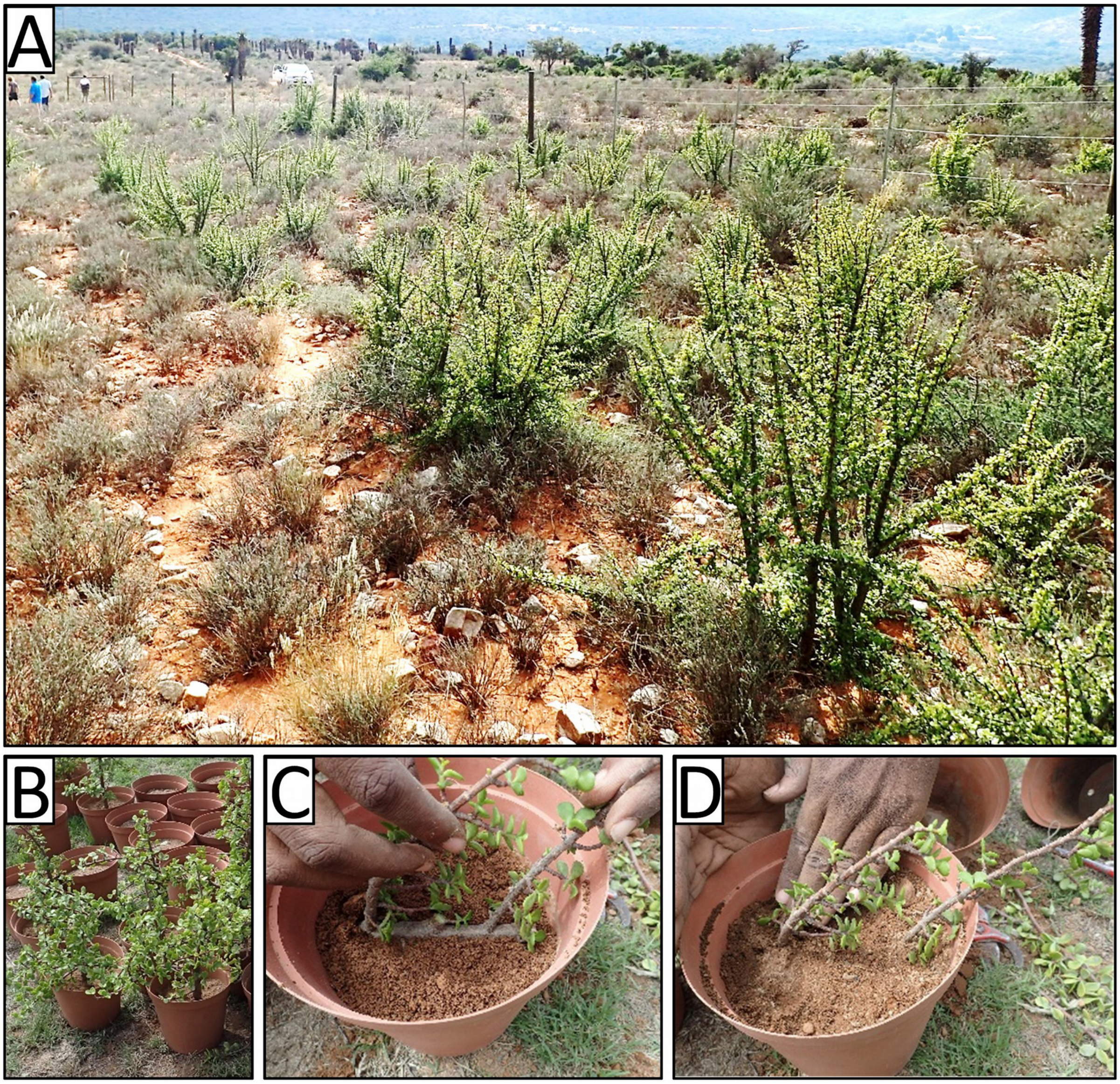

Figure 1. A: Successfully established cuttings of *Portulacaria afra* (commonly known as spekboom) in the Baviaanskloof region. These plants were established using the vertical planting protocol. **B:** Example of the vertical planting orientation, versus **C & D**: the horizontal planting orientation.

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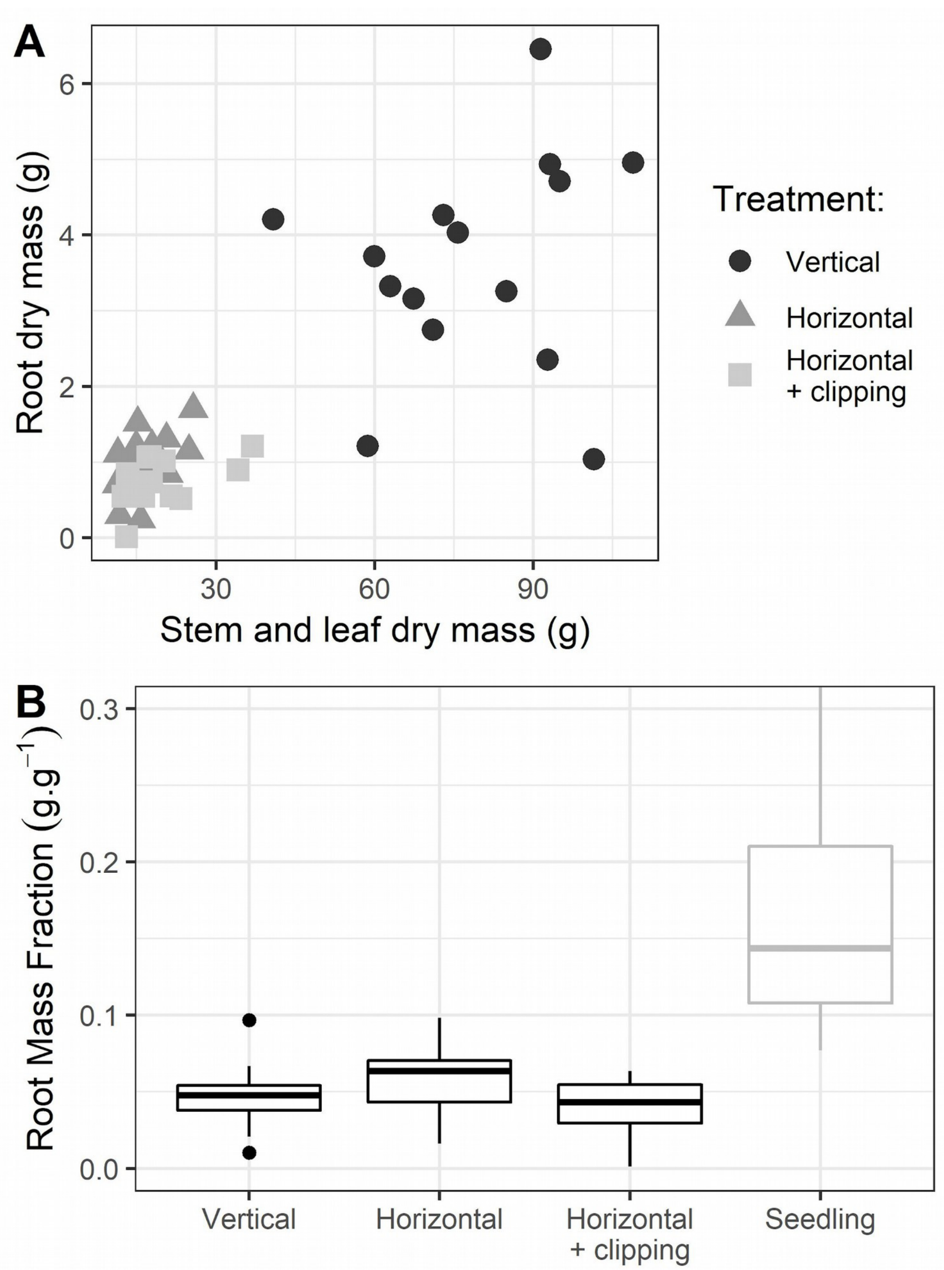

Figure 2. A: Comparison of the dry biomass of stem and leaves versus roots of *Portulacaria afra* cuttings (60 days after planting) across three planting treatments (n=15 per treatment): vertical planting (standard protocol), horizontal planting of primary stem, and horizontal planting of the primary stem with the above-ground side stems clipped at 15 cm). The vertical treatment had significantly higher dry root biomass than the other two treatments ($F_{2,42}$=125.70, p<0.001; H=29.38, p<0.001). **B:** Root mass fraction (i.e. root dry mass/total plant dry mass). Root mass fraction did not significantly differ amongst treatments ($F_{2,42}$= 2.72, p = 0.08; H=4.81, p=0.09). Root mass fraction of seedlings (from unpublished data, Guralnick and Potts) is shown for discussion purposes. See text for further details and supplementary material for images of the treatments.

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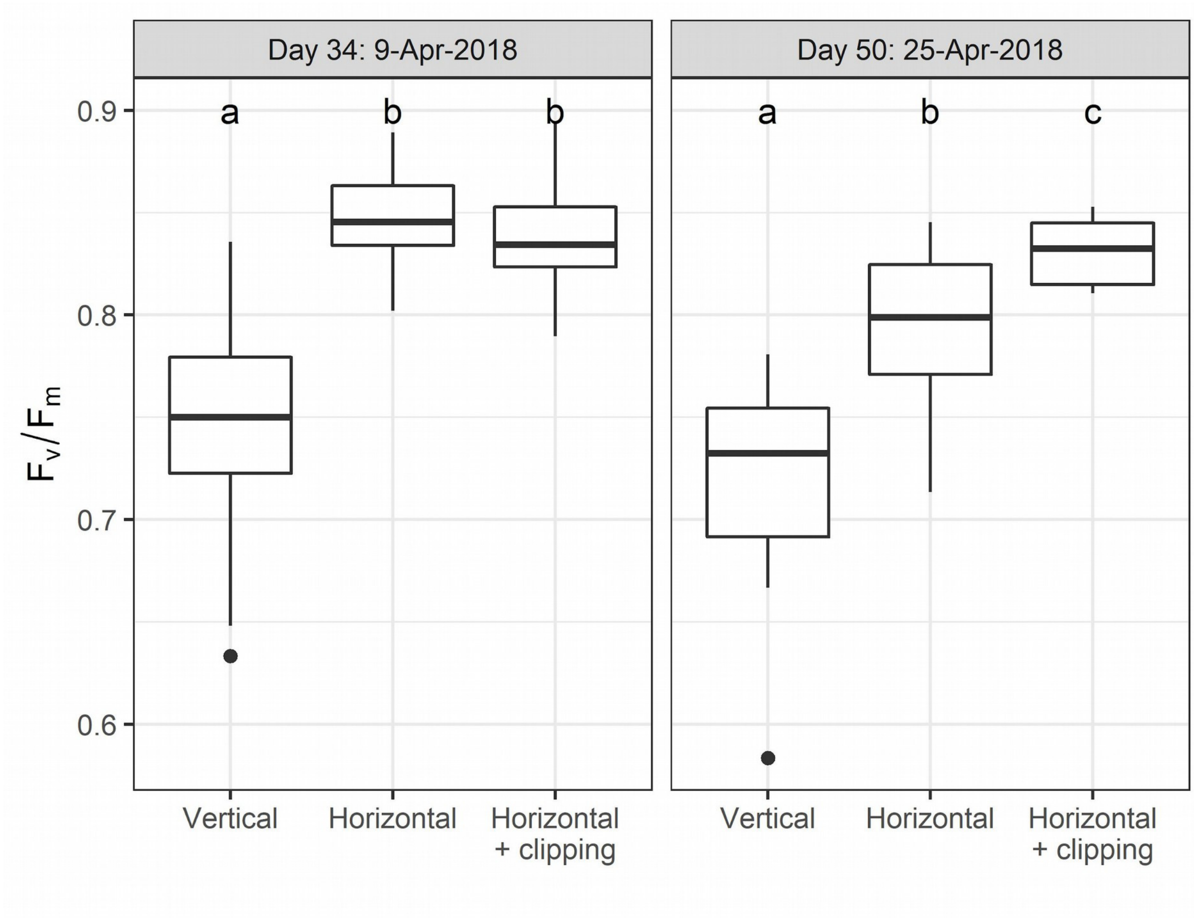

Figure 3. Photosynthetic efficiency (F_v/F_m) across the planting orientation treatments (15 plants with 5 measurements per plant) at two points during the experiment (total days=60). There were significant differences in means on both sampling days (Day 34: $F_{2,42}$=29.7, p<0.0001, H=25.1, p<0.0001; Day 50: $F_{2,42}$=34.2, p<0.0001, H=29.2, p<0.0001). Significant differences amongst treatments are shown with dissimilar superscripts (parametric Tukey and nonparametric Dunn post-hoc tests were congruent).