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Effects of water stress and mycorrhizas on rooting of stem cuttings of three dryland and semi-arid tropical tree species

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ABSTRACT
Objectives: Some species in genus *Albizia* spp. are not easy to propagate through seeds, as on maturity their seeds are quickly damaged by insects. This work was aimed at studying the effects of water stress and mycorrhizas on propagation, through rooting leafy-stem cuttings of *Albizia gummifera, A. lebbeck* and *A. schimperiana*.
Methodology and Results: Seedlings of dryland species, *Albizia gummifera*, *A. lebbeck* and *A. schimperiana* were grown under water stressed or Non-water stressed conditions. Half of the seedlings in each of these treatments were further grown with or without mycorrhizas. All the three *Albizia* species so treated were propagated through rooting their single-node cuttings in a non-mist propagator. Cuttings from water-stressed seedlings had shorter durations in rooting and higher rooting percentages. All seedlings grown with live mycorrhizal inoculum were observed to have mycorrhizas. On planting, rooted cuttings from water-stressed, mycorrhizal seedlings, grew better. Stumps remaining after harvestings shoots for taking cuttings produced new coppice shoots.
Conclusion and application of findings: The *Albizia* spp. *A. gummifera*, *A. lebbeck* and *A. schimperiana*, have been mass propagated through rooting leafy-stem cuttings in a non-mist plant propagator. This method can be used to mass-propagate any other tree that cannot be easily propagated through seeds. The use of a non-mist plant propagator to mass-propagate plants can be said to be appropriate to grass-root communities. In that, the system does not require electricity or piped water. The better performance in rooting shown by the water-stressed *Albizia* seedlings could be an adaptation to survival in the semi-arid/dryland conditions. The same could be said on the better shoot height and dry weight of the planted rooted-cuttings obtained from water-stressed, mycorrhizal seedlings. Coppice shoots are known to behave just like seedlings. As such, they can be used to obtain leafy cuttings for rooting. Thus, mass-propagation of a tree species can be perpetuated through rooting of cuttings harvested from coppice shoots.
Key words: *Albizia*, water-stress, rooting cuttings, plant propagator, mycorrhizas, dryland, semi-arid

INTRODUCTION

Species of *Albizia* (Fabaceae, Mimosoideae) play several important roles in drylands and semi-arid areas of the tropics. They are used as animal fodder (Prinsen 1988; Komwihangilo *et al*. 1995; Mtengeti & Mhelela 2006; Lowory *et al* 1998; Yisehak & Duguma 2011); in improving soil quality (Olojugba &

Oke 2015; Oyebamiji *et al* 2016), as well as wind breakers (Stigter *et al*. 2002; 2003) and in restoration of woody vegetation in drylands (Kigomo 2004). In tropical drylands and semi-arid areas, environmental degradation has been developing as result of over-grazing, over-cultivation, cutting trees for firewood, building poles, among other uses. Afforestation combined with revegetation is the dominant focus for landscape planning, designed to promote the recovery of the goods and services provided by these ecosystems (Kiyono *et al.*2007). Indeed, it is always advantageous if a graded ecosystem is rehabilitated with local indigenous flora. To do this, availability of sufficient planting stocks of the indigenous trees and shrubs is essential. Most tropical trees are known to be obligately mycorrhizal, as they depend on them for survival as observed by Högberg & Nylund 1981; Högberg 1982; Högberg & Pierce 1986; Högberg 1990; Munyanzinza 1993; Högberg & Alexander 1995 and Ngulube *et al.* 1995. Mycorrhizal associations positively affect plant establishment, growth and health. They are beneficial, and in some instances essential (Jackson & Mason 1984). The best-known benefits of mycorrhizal associations include enhanced uptake of water and mineral nutrients, especially phosphorus and nitrogen (Bowen 1973). For forest trees, mycorrhizal associations are essential to prevent the failure of plantations (Marx 1980). For seedlings, mycorrhizal associations are very essential for their survival and growth in the nursery, and for establishment and better growth once out-planted (Castellano & Molina 1989). The above statement is also applicable to rooted cuttings, as has been observed for rooted cuttings of the shrub *Sorbus aucuparia* (Morrison *et al*. 1993), beech (*Fagus sylvatica*) and spruce (*Picea abies*) (Schmitz 1993; Genere *et al.* 1994). One of the setbacks in vegetating semi-arid and drylands is lack of planting materials and survival of the planted material. This work was aimed at studying the effects of water-stress and mycorrhizas on the rooting of stem cuttings of the dryland/semi-arid species *Albizia gummifera, A. lebbeck* and *A. schimperiana*.

MATERIALS AND METHODS

Rooting of cuttings of water-stressed and Non-water stressed seedlings of Albizia species: In order to have water-stressed and Non-water stressed seedlings of *Albizia*, a total of 144 four months old seedlings, that is, 48 seedlings for each of the three *Albizia* species, *A. gummifera, A. lebbeck* and *A. schimperiana* were used. Half of the pots of the plants in each species were watered to field capacity twice a week (Tuesdays and Fridays). However, during sunny and hot weeks temperatures in the tropicalized glass house reached 28 to 33˚C. During such weeks, an additional watering of the same volume of water was administered on Mondays and Thursdays. Water drained through the pots after each watering indicating that the soil in such pots had reached near to field capacity. The water-stressed plants were given ~60% less water. The water-stressed plants did not reach field capacity after such watering, as excess water did not drain off from the pots. The volumetric water content of the soil was measured before and after watering with a moisture meter (HH2, Delta-T Devices., Ltd.). Plant height was recorded weekly and root collar diameter (RCD) was measured 3 times when the seedlings were 5, 6 and 7 months old, using a calliper with a digital display (Model CD-6, Mitutoyo Corporation, Japan). When seedlings had grown for 7 months, leafy stem-cuttings each 5 cm long were taken from 24 seedlings (clones) of each species. The basal diameter of each cutting was measured using a calliper with a digital display (Model CD-6, Mitutoyo Corporation, Japan). The compound leaves on each cutting were trimmed to remain with 2-4 pinnae such that on each cutting the total leaf area did not exceed 60 cm^2. The basal end of each cutting was dipped in the commercial rooting powder seradix-2 (Rhône-Poulenc Agriculture Ltd. Essex, England) containing 0.3% w/w 4-indol-3yl-butiric acid. Excess powder was tapped off, before inserting the cutting in non-mist propagators (Leakey *et al.* 1990) which were held inside a tropicalized glass house. The bottom of each propagator was filled with quartz grit (0.01 – 12.7 mm) rooting medium and filled with water to reach 7 cm below the surface of the quartz grit. The bed temperature of the propagators was maintained at between 25 and 30˚ C. Cuttings from each seedling were placed in node order in the non-mist propagators. In total, 6 propagators (blocks) were used, and in each propagator, cuttings from an equal number of water-stressed and non-water stressed seedlings (clones) were inserted. The proportion of leaf loss, number of new shoots and their total length was assessed twice a week for each cutting until it was recorded as rooted or dead. A cutting was considered rooted if it had at least one root whose length was 1.0 mm or more, and dead if the stem of the cutting was completely rotted. From the remaining 2 seedlings (clones) of each species in each block, leafy cuttings each 5 cm long were also taken. The basal diameter each of these cuttings was measured using a calliper with a digital display (Model CD-6, Mitutoyo Corporation, Japan), and the number of leaflets on each cutting was trimmed so that their total area does not exceed 60 cm^2, Each of these cuttings was separately packed in labelled paper bag and dried in an oven at 80 ˚C for 7 days to obtain dry weights data for day zero. After harvesting cuttings, the pots with their remaining stumps, were returned to the tropicalized glass house in which they had been growing and the two watering regimes maintained. That was to observe re-growth of coppice shoots on the stumps.

Effects of mycorrhizas to water stress tolerance of the Albizia species. : For this work, 4 months old seedlings of the trees *Albizia gummifera*, *A. lebbeck* and *A. schimperiana*, were singly potted into bigger pots of size 10.5 x 10.5 x 11 cm. During potting, half of the plants of each species were inoculated with live mycorrhizal inoculum, while the other half of the plants of each species were inoculated with sterilized mycorrhizal (dead) inoculum. Live mycorrhizal inoculum consisted of 30 g of a mixture of soil and roots of the same plant species. The bulk inoculum had been taken from the top 15 cm rhizosphere of 5 different trees of the same species from Iringa and Songea in Tanzania. During inoculation, each pot had its seedling hygienically hand uprooted. A hole was then made in the soil remaining in the pot, and 30 g of the live mycorrhizal inoculum placed in the hole. The seedling was then inserted back into the pot, with its roots resting on the live mycorrhizal inoculum. The stem portion of the seedling in the pot was then covered with the soil mixture. Half of the seedlings of each species that comprised the control set were inoculated with dead mycorrhizal inoculum. This type of inoculum was the same as the one used for the live mycorrhizal sets, except for the fact that, now it is dead inoculum because of sterilizing the live mycorrhizae inoculum in an autoclave at 121 ˚C for two 1-hour periods, with a 24 hour period between the autoclaving. All pots, each with a single plant were randomly placed on a bench in a tropicalized glass house. For one month, each pot was watered with 150 ml filter sterilized tap water twice a week, on Tuesdays and Fridays. After one month of the above watering treatment, water stressing of all the plants under the mycorrhizal effects experiment was begun. From this day, all plants were watered with Ingestad solution (Ingestad 1979) so made that, it had 5 ppm phosphorus and 31.25 ppm nitrogen. Bigger plants that were 10 cm or taller, were given 100 ml of the Ingestad solution on Tuesdays and Fridays. Smaller plants, less than 10 cm in height, were given 60 ml Ingestad solution on the same days as the taller ones. During sunny and hot weeks, temperatures in the tropicalized glass house reached 28 to 33 ˚C. During such weeks, the plants were extra watered with filter sterilized tap water on Mondays and Thursdays. The extra watering consisted giving 50 ml of the filter sterilized tap water to the bigger plants and 30 ml to the smaller plants. Plant height was measured using a ruler, while root collar diameter (RCD) was measured using a calliper with a digital display (Model CD-6, Mitutoyo Corporation, Japan). The height and RCD were recorded after every two weeks. After 4 months of water-stress treatment, all plants were harvested and the root system of each plant washed clean of all soil and debris. Observations for mycorrhizal infections in the roots of the plants were carried out. The shoot of each plant was oven dried at 80 ˚C for 7 days, to obtain shoot dry weight.

RESULTS

Growth of the water stressed and Non-water stressed Albizia plants and rooting of their cuttings: The water stressing did affect growth of the plants under this treatment. The water stressing caused the plants to completely stop growing from day 34 (Fig 1.). On day 60, when the height recording was terminated, the water-stressed plants did have significantly smaller root collar diameters (Fig. 2).

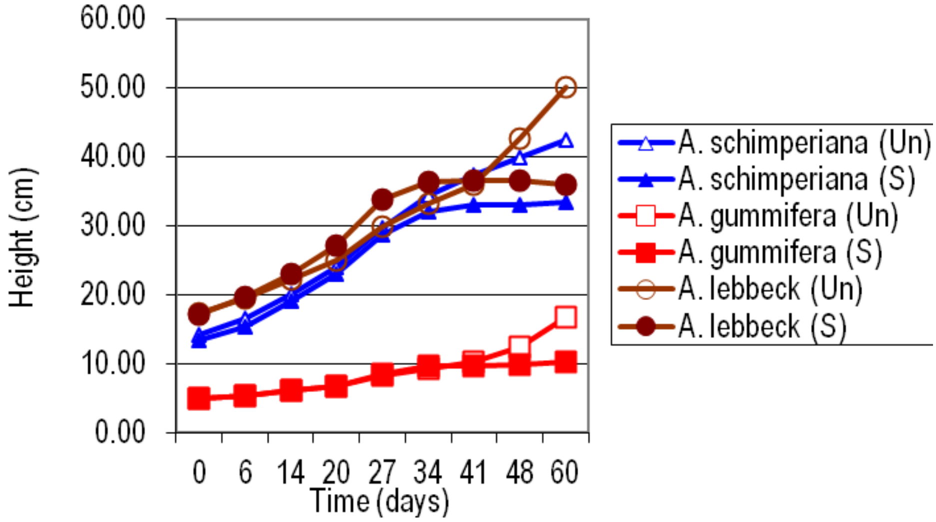

Figure1: Height of water stressed (S) and Non-stressed seedlings of *Albizia* species.

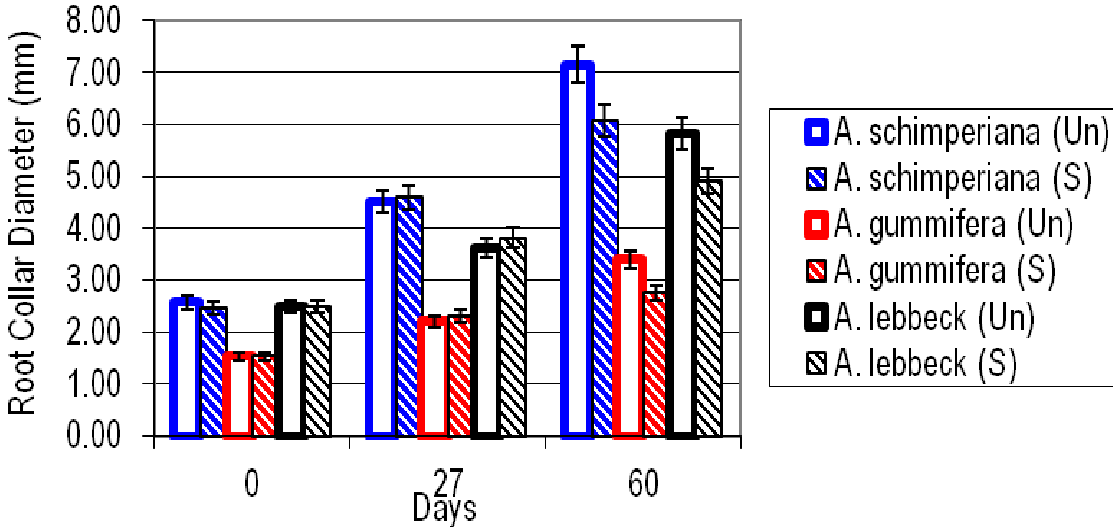

Figure 2: Root collar diameter (RCD) of water stressed (S) and Non-stressed (Un) seedlings of the three *Albizia* species.

Figure 3: Rooted cuttings of *A. gummifera* (left), *A. lebbeck* (middle) and *A. schimperiana* (right)

Albizia gummifera had significantly slower growth rate (RCD) compared to both *A. lebbeck* and *A. schimperiana*. Five months after germination when the water stress treatment was applied the *A. gummifera* seedlings were significantly shorter ($p<0.001$) with a mean height of 8.3 cm compared to 29.2 and 31.8 cm for *A. schimperiana* and *A. lebbeck* respectively. There was a significant difference ($p<0.001$) in root collar diameter between all three species ranging from 2.26 mm for *A. gummifera* to 4.55 for *A. schimperiana* with *A. lebbeck* having intermediate RCD at 3.73 mm. There was no significant difference in height or root collar diameter between the seedlings allocated to the water stressed or non-water stressed treatments within each species when treatments were started. After 5 months, when the cuttings were harvested from stock plants, water-stressed seedlings of all the three species had stopped growing (Fig. 1) and were significantly shorter than the non-water stressed seedlings ($p<0.004$). There was also a highly significant ($p<0.001$) difference in height between all three species. There was a similar trend in root collar diameter with water stressed plants having an average of 4.58 mm compared to5.46 mm for non-water stressed seedlings. The root collar diameter of *A. gummifera*, *A. lebbeck* and *A. schimperiana* seedlings were 3.08, 5.37 and 6.61 mm respectively. Within a species, there were no significant differences between water-stressed and non-water stressed treatments concerning morphological parameters of the cuttings at harvest. The morphological traits were however, highly significantly different between species. Cuttings harvested from *A. gummifera* seedlings had smaller diameter and consequently were significantly lighter than the other two species (~50%). *A. schimperiana* cuttings were significantly heavier than *A. lebbeck*, but the differences were only ~20% and were again a consequent of stem dimensions rather than leaves. Some rooted cuttings of the three *Albizia* species are shown in Figure 3. There were significant differences in final rooting percentages between species (ANOVA $p<0.001$) but not treatment ($p=0.38$). The final rooting percentage of *A. lebbeck* were 79% and 77% for cuttings collected from water-stressed and non-water stressed stock plants respectively, while *A. gummifera* cuttings achieved 77% and 73%. These species were significantly more successful than *A. schimperiana*, which reached only 50% and 39% rooting after 11 weeks in the propagators (Fig. 4).

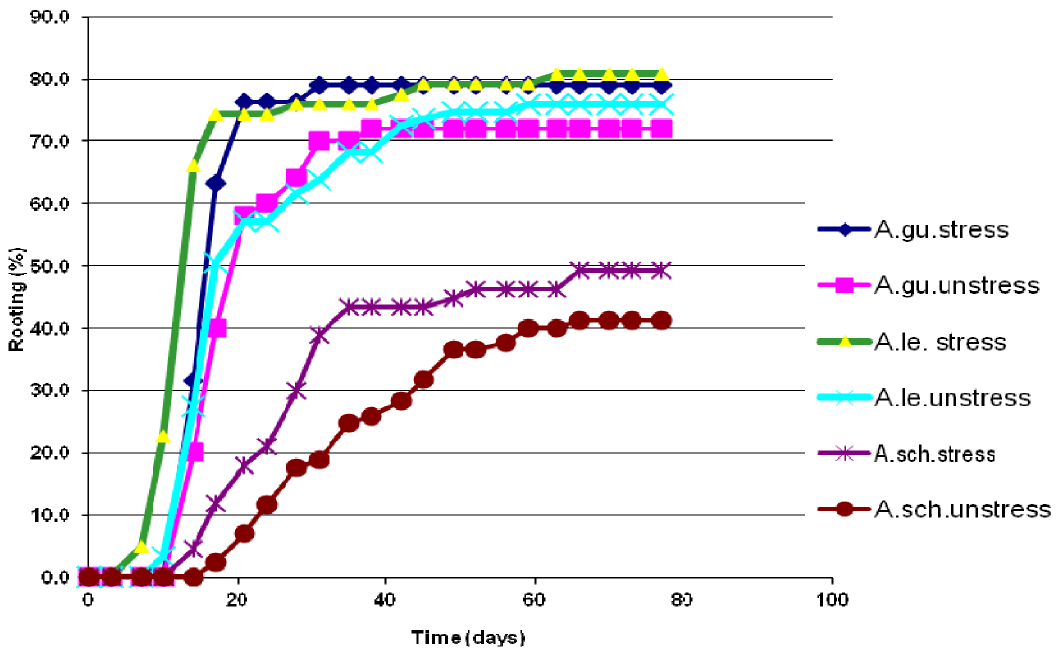

Figure 4: Percent rooting of cuttings of the three *Albizia* species harvested from 5 months old water stressed (stress) and Non–water stressed seedlings

There was a consistent difference in the rate of rooting between the water stressed and the non-water stressed treatments. Cuttings harvested from water-stressed stock plants produced roots faster with 50% of the rooting response averaged across all the three species occurring by day 17, compared to day 21 for the non-water stressed treatment (Table 1). Cuttings harvested from *A. schimperiana* seedlings were also noticeably slower to produce roots compared to the other two species.

Table 1: Days to 50% rooting response

	Water Stressed	Non-water stressed
A. Lebbeck	11.0	15.25
A. Gummifera	14.5	16.5
A. Schimperiana	26.0	32.0

Mycorrhizas on the three Albizia species: All the three *Albizia* species were observed to possess vesicular arbuscular mycorrhizae (VAM). Some details of mycorrhizas in the roots of the *Albizia* seedlings are shown in figures 5 to 13.

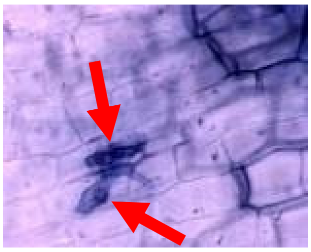

Figure 5:
Appressoria (see arrows) of mycorrhizal fungus penetrating root-epidermal cells of *Albizia gummifera* seedling (X 40)

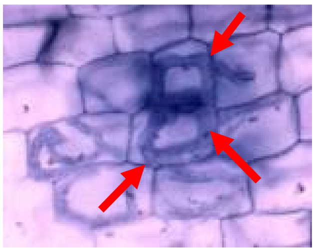

Figure 6:
Coiled hyphae (see arrows) of mycorrhizal fungus in the root cortical cells of *Albizia gummifera* seedling (X 40)

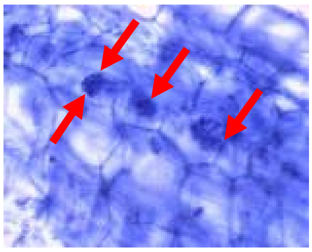

Figure 7:
Arbuscules (see arrows) of mycorrhizal fungus in the root cortical cells of *Albizia gummifera* seedling (X 40)

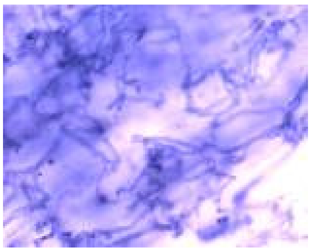

Figure 8:
Hyphae of a mycorrhizal fungus in the root cortex of *Albizia lebbeck* (X 20)

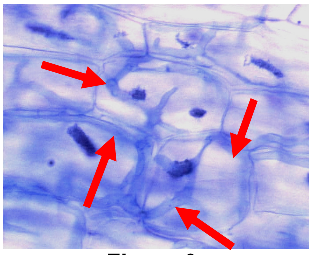

Figure 9:
Extensive hyphal spread (see arrows) of a mycorrhizal fungus in the root cortical cells of *Albizia lebbeck* seedling (X 40)

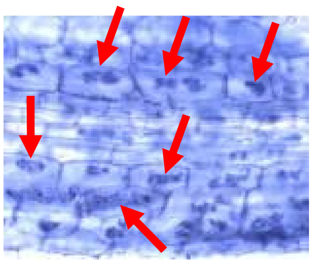

Figure 10:
Extensive arbuscules (see arrows) developed in the cortical cells of *Albizia lebbeck* seedling root (X 20)

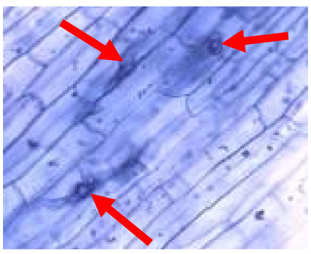

Figure 11:
Appressoria (see arrows) of mycorrhizal fungi penetrating the epidermal root cells of *Albizia schimperiana* seedling (X 40)

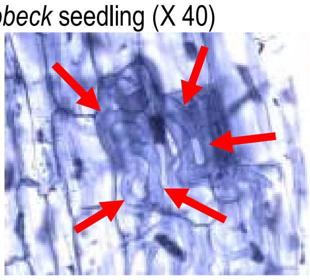

Figure 12:
Coiled hyphae (see arrows) of mycorrhizal fungus just below root-epidermal cells of *Albizia schimperiana* seedling (X 40)

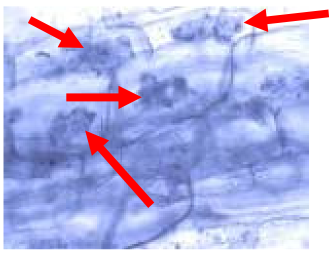

Figure 13:
Arbuscules (see arrows) in the root cortical cells of *Albizia schimperiana* seedling (X 40)

Effects of mycorrhizas on water stress tolerance: Rooted cuttings of all the three species of *Albizia* potted with live mycorrhizal inoculum had higher shoot height and dry weight, compared to those potted with dead mycorrhizal inoculum (Fig. 14). On the other hand, root collar diameters were not significantly different between the two mycorrhizal treatments.

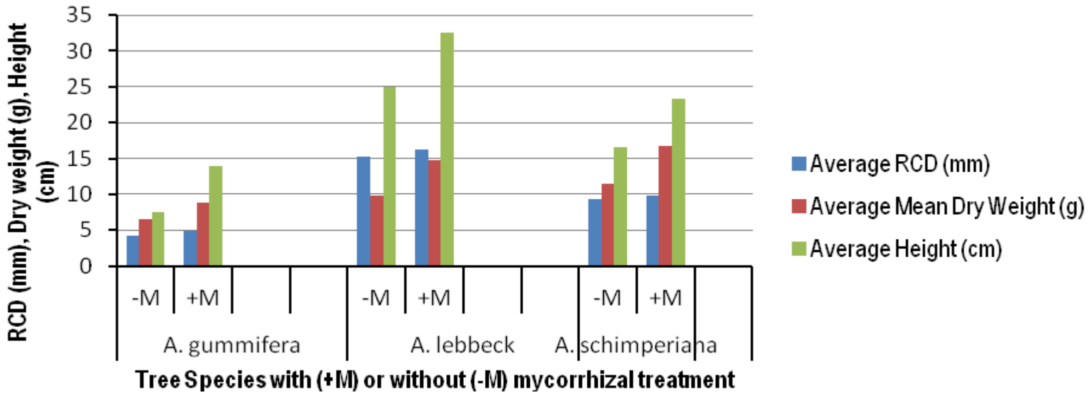

Figure 14: Root Collar Diameter (RCD) (mm), Dry weight (g) and Shoot Height (cm) of *A. gummifera*, *A. lebbeck* and *A. schimperiana* plants potted with live (+M) or dead (-M) mycorrhizal inoculum

Coppicing of stumps left after harvesting shoots for obtaining cuttings: After harvesting of shoots for obtaining cuttings for rooting, stumps of the plants left to grow in the tropicalized glass house produced coppice shoots (Fig. 15). In this work however, studies on the coppice shoots were neither continued, nor quantified.

Figure 15: Coppicing stumps

DISCUSSION

It has been shown in this work that, the semi-arid trees *Albizia gummifera*, *A. lebbeck* and *A. schimperiana*, can be mass propagated through rooting of leafy stem cuttings using a non-mist propagator. Despite being negatively affected in terms of plant height (Fig. 1) and root collar diameter (Fig. 2), water stressed plants of the three *Albizia* spp. had their leafy stem cuttings rooting better than well-watered plants. This was in terms of final percent cuttings rooted (Fig. 4) and shorter period to 50% rooting response (Table 1). The better performance in rooting shown by leafy stem cuttings from water stressed plants could be an explained as an adaptation to overcome the harsh water conditions in the semi-arid areas to ensure survival of the species. Other species of *Albizia* have also been reported to be propagated through rooting of their stem cuttings. They include *A. adianthfolia*, *A. ferruginea*, *A. zygia* (Gerald 2000) and *A. falcataria* (de Muckadell 1984). However, for *A. gummifera*, *A. lebbeck* and *A. schimperiana*, this is a first report. *Albizia* species can be propagated through seeds (Lowory *et. al*. 1998, Mbuya *et. al*. 1994, Senbeta *et. al*. 2002). However, the problem with this method is that, on maturity the seeds of the *Albizia* spp. are quickly damaged by insects (Mbuya *et. al*. 1994). As such, propagating the *Albizia* species vegetatively through rooting of their leafy stem cuttings becomes the best alternative. Rooted cuttings of the three *Albizia* species potted with live mycorrhizal inoculum, did form mycorrhizal associations. This was observed in the roots of the three *Albizia* species (Fig. 5 to 13). The mycorrhizal associations had significantly positive effects on the three *Albizia* species studies in this work. That was in terms of plant height, root collar diameter and dry weights (Fig. 14). Other workers have previously shown that, in nature, growth of the seedlings of the plant species *A. gummifera*, *A. lebbeck* and *A. schimperiana* and other *Albizia* species are indeed positively affected by mycorrhizas (Osonubi *et. al*. 1991; Sharma *et. al.* 2001; Wubet *et. al*. 2003; Tuheteru & Husna. 2011). Indeed, in all these previous reports, seedlings of the plants were used. On the other hand, in this work, rooted stem cuttings of the *Albizia* species have been used. It can be generalised that, when it comes to mycorrhizal effects, seedlings and rooted stem cuttings of *A. gummifera*, *A. lebbeck* and *A. schimperiana*, behave in a similar manner. One of the setbacks in re-vegetating semi-arid areas is lack of sufficient planting materials. This work has demonstrated that, this problem can be alternatively overcome through mass-propagating the semi-arid trees through rooting of their stem cuttings. In addition, this work has demonstrated that, mycorrhizal associations have very positive influences on the growth of the propagated plants. As such if the propagated plants have to survive better on planting in the field, mycorrhizal fungi have to be inoculated onto the propagated plants on planting in the field. Stumps left after harvesting shoots for making cuttings produced new coppice shoots when left to grow. Several workers have observed dryland/semi-arid trees coppicing in nature. The species include those growing in dry miombo woodlands (Syampungani *et al* 2017). *Albizia* species studied in this work are also known to coppice in nature, as reported for *A. gummifera* by Maroyi (2007) and Yisehak & Duguma (2011); for *A. lebbeck* by Saleem & Singh (1995) and Parrotta (2014); and for *A. schimperiana* by Alelign (2007) and Assefa *et al* (2017). With respect to rooting, coppice shoots are known to behave just like seedlings (Longman & Wilson 1998; 2002; Longman *et al* 2003; Husen 2011; Wendling & Brondani 2015). Thus, mass-propagation of a tree species can be perpetuated through rooting of its coppice shoots.

CONCLUSION

Three dryland/semi-arid tree species in genus *Albizia* have been mass-propagated through rooting of their stem leafy cuttings in a non-mist propagator. This method could be used to mass-propagate any other tree that cannot be easily propagated through seeds. In line with adaptation to semi-arid/dryland conditions, water-stressed individuals of the trees *A. gummifera*, *A. lebbecck* and *A. shimperiana* rooted better than Non-stressed ones. The use of a non-mist plant propagator to mass-propagate plants can be said to be appropriate to grass-root communities. In that, the system does not require electricity or piped water. Another adaptation to semi-arid conditions observed in this work is the better growth of water-stressed rooted cuttings possessing mycorrhizas when planted. It can as well be said that, possessing mycorrhizas is a good attribute enabling survival of propagated semi-arid/dryland plants when planted in the field.

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