Full Length Research Paper

Effect of growth media and fertilizer application on biomass allocation and survival of *Uapaca kirkiana* Müell Arg seedlings

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Accepted 13 August, 2007

Uapaca kirkiana Müell Arg is one of the few recognized African fruit trees earmarked for conservation and domestication in southern Africa. However, efforts to raise seedlings of this species have been frustrated by the slow growth and high seedling mortality. Therefore, the objective of this study was to assess the effect of potting mixture, soil and foliar fertilizer application on plant growth, biomass allocation and survival of *U. kirkiana* seedlings in the nursery. Growth in height and diameter was best in plants with root to shoot ratio of <1 or 2.5-4. Growth in height and diameter significantly differed (P<0.01) with treatment main effects and interactions. The best growth was recorded in the treatment combination consisting of unsterilized forest soil + soil and foliar application fertilizer. The probability of plant mortality was significantly higher (P<0.01) in the potting mixture where saw dust was added (mean 0.47) than in the mixture without saw dust (mean 0.12). Mortality was also significantly higher (P<0.05) in unsterilized soil (mean 0.30) than sterilized soil (mean 0.13). Potting mixtures amended with soil-applied fertilizer had lower probability of plant mortality compared to those without. Disease incidence and seedling survival were related to biomass allocation in a curvilinear manner. It is concluded that survival of *U. kirkiana* seedlings in the nursery is a function of disease incidence, plant growth and biomass allocation, which in turn are functions of the growth medium and nutrient availability.

Key words: Biomass allocation, growth, miombo, mortality, Uapaca kirkiana.

INTRODUCTION

Uapaca kirkiana Müell Arg (Euphorbiaceae) is one of the few recognized African fruit trees with economic potential and extensive local use (Maghembe et al., 1994). It is typically a species of the miombo woodland—the largest continuous dry deciduous forest in the world extending across much of central, eastern and southern Africa (Campbell et al., 1996). *U. kirkiana* has been ranked by farmers, scientists and consumers as the top most preferred fruit tree in southern Africa (Maghembe et al., 1998), and it has been earmarked for conservation and domestication (Akinnifesi et al., 2006; Mwase et al., 2006). Although opportunities for commercial exploitation exist, it is underutilized because much of its ecology is unknown and cultivation

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efforts have been limited. Large-scale commercial production of wine from its fruits initiated in Zambia and Malawi have been discontinued due to the erratic availability of fruits.

As forests and conservation areas are being degazetted in favour of agricultural activities and settlements to accommodate growing populations, a drive towards domestication and on-farm planting of *U. kirkiana* is a feasible option for conservation and management of the species (Mwase et al., 2006). However, efforts to raise its seedlings have been frustrated by the slow growth and high seedling mortality in the nursery in Malawi (Mhango, 2000) and Zambia. Survival after out planting has also been reported to be as low as 20% in Zambia (Mwamba, 1989) and 67% in Malawi (Mhango and Akinnifesi, 2001).

There has been repeated failure in attempts to raise seedlings of *U. kirkiana* over the years in eastern Zambia

and the causes are still not yet known. Over 4429 seedlings were raised in December 1999 using a potting mixture recommended for U. kirkiana (Mwamba, 1995) containing 75% miombo forest soil and 25% sand. By November 2000, 100% mortality was recorded. At the time direct insolation at the nursery site was suspected to be the cause of mortality, as U. kirkiana seedlings are adapted to partial shade of the mature trees in the miombo woodlands (Ngulube et al., 1995). Therefore, the nursery was shifted to a shaded location, and 3080 seedlings were raised in a screen house in December 2000. A potting mixture recommended for U. kirkiana in Malawi (Mhango, 2000) consisting of 75% miombo forest soil + 25% sawdust was used. Fungicides were applied to protect the seedlings from fungal diseases. However, less than 50% survived to reach a stage suitable for grafting. This led to the suspicion that insufficient amount of mycorrhizae innoculum in the miombo soil from Msekera was the cause of seedling mortality. Therefore, in December 2001, soil from different natural U. kirkiana stands were collected, and a potting mixture consisting of 75% forest soil + 25% saw dust was constituted to raise a total of 3683 seedlings. Again only 3% survived to reach a stage suitable for grafting. At Makoka research station in Malawi, U. kirkiana has shown poor seedling growth but generally less mortality has been recorded (Mhango, 2000) than the levels observed at Msekera in Zambia. Seedling survival becomes critical especially with seedlings raised as rootstock material for vegetative propagation as the seedlings have to stay in pots for a period not less than nine months before they reach a suitable stage for grafting.

Mortality in seedlings could be attributed to different factors including diseases, inappropriate growth media, nutritional deficiencies (Mhango, 2000; Swai, 2002) and lack of mycorrhizae (Högberg, 1982; Mwamba, 1995; Ramanankierana et al., 2007). However, it is not well known to date as to what causes the slow growth and high seedling mortality in U. kirkiana. Various stress factors including soil moisture (Nadelhoffer et al. 1985), nutrient availability (Gower, 1987) and soil texture (Gerhardt and Fredriksson, 1995) determine biomass allocation, seedling growth and survival. Knowledge of how seedlings allocate their biomass in response to stress and management practices may aid in understanding plant growth and survival in the nursery and performance in the field. Therefore, the objective of this study was to assess the effect of potting mixture, soil and foliar fertilizer application on plant growth, biomass allocation and survival of U. kirkiana seedlings in the nursery.

MATERIALS AND METHODS

The study was conducted at Msekera Research Station $(13^{\circ}39'S, 32^{\circ}34' E, altitude 1025 m)$ in eastern Zambia. The treatments consisted of a factorial combination of three potting mixtures (unsterilized forest soil, sterilized forest soil and a mixture of 75% unsterilized forest soil and 25% saw dust), soil applied fertilizer (with and with-

out Compound D), and a foliar applied fertilizer (with and without Folifert^M). Soil sterilization was aimed at destroying soil-borne diseases. The experimental unit consisted of 1210 individual potted seedlings raised from seeds of *U. kirkiana* planted in polyethylene pots in December 2002. Each potted seedling constituted a treatment combination, and pots were arranged in a completely randomized design.

Forest soil was collected from a natural forest stand of U. kirkiana and sieved to remove unwanted materials. For the sterilized soil potting mixture, a heap of the sieved forest soil was placed under a plastic cover and steam from water boiling in a drum was directed into the heap using a hose pipe. The steaming took three hours achieving soil temperature of about 100°C. Part of the unsterilized soil was mixed with saw dust to constitute 75% soil and 25% saw dust. Each potting mixture was filled in polythene pots measuring 37 cm height and 21 cm diameter. One seed was directly sawn into each pot. Before sowing the endocarp was removed and seeds were cleaned. The treatment with soil applied fertilizer was supplied with Compound D, a slow-release fertiliser composed of N =100 g kg , P = 90 g kg⁻¹, and K = 80 g kg⁻¹. This was applied at the rate of 5 g per pot applied every month during the first nine months of the experiment. The foliar fertilizer was Folifert[™] consisting of 41.5 g/l N, 58.2 g/l P, 1409 mg/l Zn, 1409 mg/l Cu, 2818 mg/l Fe, 1409 mg/l Mn, 198 mg/l Mo, 2818 mg/l B, 2.2 mg/l Auxin and 0.006 mg/l Cytokinins. The Folifert was applied by spraying it uniformly on the foliage of each plant at the time when the soil applied fertilizer was applied.

Seedling emergence was recorded one month after planting. Measurements of seedling growth variables and survival were taken on 20th October 2003, 11 months after planting. Out of the 1210 seedlings raised a total of 260 seedlings have survived and these were available for measurement. Height was measured from the root collar to the tip of the shoot. Root collar diameter was measured using a calliper. In U. kirkiana, rootstock diameter is an important factor for successful union of the scion and root stock (Mhango, 2000). Tap root length and dry-matter production was obtained by carefully uprooting the seedlings from the pot. The roots were thoroughly washed and each plant separated into leaves, stems, and roots. Length of the tap root was measured from the root collar to the root tip with a meter ruler. Then the shoot and root components of each seedling were placed in paper bag and dried at 65 °C for 48 h and weighed. Root to shoot ratio was calculated as the ratio of the dry weight of root to the dry weight of the shoots and leaves. Foliar disease incidence, defined as the proportion of plants showing foliar disease symptom (leaf spot, blighting or necrosis). was recorded in July and October 2003. Seedling mortality (proportion of dead plants per treatment) was recorded in October 2003.

Prior to analysis, growth and biomass data were explicitly tested for normality (Shapiro-Wilk statistic) and homogeneity of variance (Leven's test). Since the assumptions were not satisfied by the data, a linear mixed model was used to relate growth variables and biomass portioning to treatment effects. The most parsimonious model among a set of models was found using the minimum Bayesian information criterion (BIC), as BIC is typically a consistent model selector (Sileshi, 2006) compared to others criteria such as Akaike information criterion (AIC). Seedling mortality was related to the type of potting mixture, and soil and foliar application of fertilizer using generalized linear models assuming binomial error distribution. Model parameters were estimated by maximum likelihood method using the GENMOD procedure of SAS systems. The best model describing foliar disease incidence and seedling mortality using treatment combinations was selected as the one that minimized BIC. The probability of seedling mortality was then calculated from the parameter estimates of the most consistent model.

Correlations between seedling height, root number, collar diameter, shoot weight, root weight, height to diameter ratio and root to shoot ratio and seedling mortality were also conducted. Since scatter plots showed non-linear relationships between seedling growth variables and biomass allocation, polynomial regression was con-

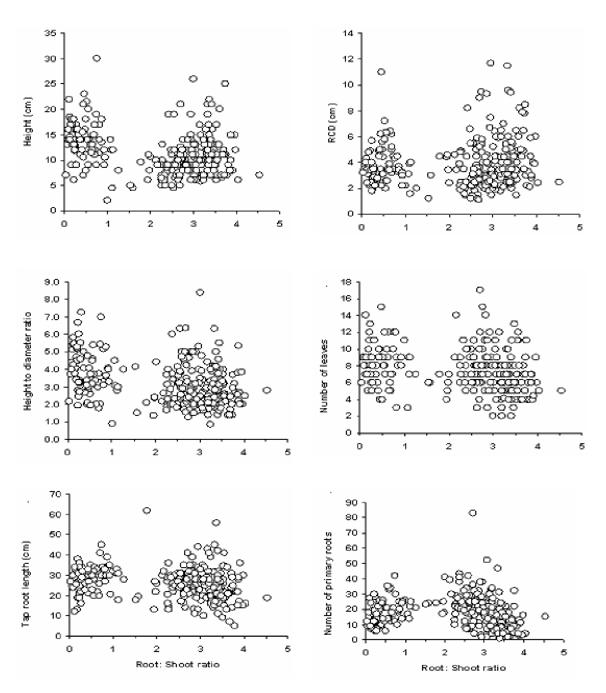


Figure 1. Scatter plots of seedling growth variables against root to shoot ratios.

ducted using the ORTHOREG procedure of the SAS system.

RESULTS

The distribution of seedling growth in height, root collar diameter, number of leaves, tap root length and number of primary roots distinctly varied with biomass allocation indicated by the root to shoot ratio (Figure 1). Fewer plants had root to shoot ratios between 1 and 2, that is, equal allocation to the roots and shoot. Growth in height and diameter was best in plants with root to shoot ratio of <1 or 2.5-4.0 (Figure 2a and b). After 11 months, seedling mortality growth in height and collar diameter (Figure 4) significantly differed (P<0.0001) with treatment main effects and the interactions. Addition of saw dust in the potting mixture and sterilization of the soil increased biomass allocation to roots than shoots (Figure 3).

Application of fertilizers to the soil and foliage reduced biomass allocation to the roots (Figure 3).

The results indicate that single variable models were poorer in terms of consistency (minimum BIC) for explaining biomass allocation and plant mortality (Table 1). Therefore,

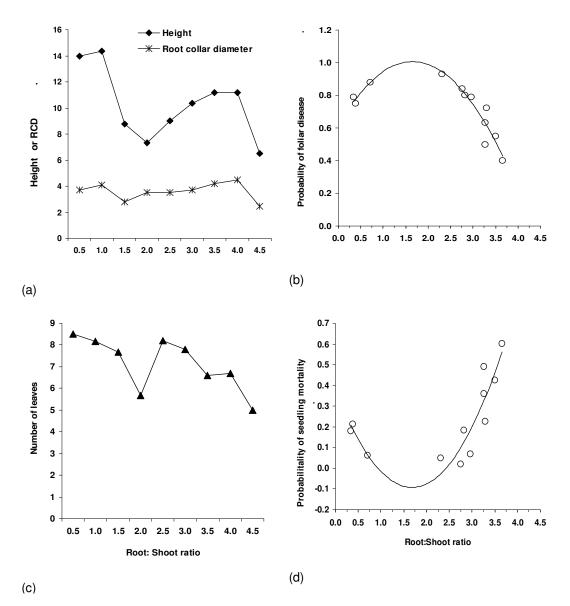


Figure 2. Effect of biomass allocation (root: shoot ratio) on height and diameter growth (a), probability of foliar disease incidence (b) number of leaves (c) and seedling mortality (d) in 11 months old *U. kirkiana* seedlings at Msekera, eastern Zambia. Probability of foliar disease incidence (Y₁) was related to root to shoot ratio (X) as: Y₁ = $0.90 + 0.63X - 0.19X^2$; R² = 0.77; F = 0.0015. Probability of seedling mortality (Y₂) was related to root to shoot ratio (X) as: Y₁ = $0.66 - 0.80X + 0.24X^2$; R² = 0.64; F = 0.0096.

parameter estimates and 95% confidence limits were computed using models with the smallest BIC score. The best growth in height (Figure 4a) and diameter (Figure 4b) was recorded in the treatment combination consisting of unsterilized forest soil + soil and foliar application fertilizer, while the poorest growth was in unsterilized forest soil mixed with saw dust foliar fertilizer. Seedlings had longer tap roots in potting mixtures without saw dust whether fertilizer was applied or not (data not shown). Seedlings grown in unsterilized forest soil mixed with saw dust developed fewer lateral roots. Seedlings grown in sterilized soil (with or without fertilizer application) developed the largest number of lateral roots. Height to diameter ratios were larger in the treatment combination consisting of unsterilized forest soil without saw dust either with or without fertilizer application. The root to shoot ratio was largest in seedlings raised in potting mixtures containing sawdust, while the smallest was in potting mixtures without sawdust (Figure 3, 4c).

Roots were developing at nearly three times the rate of the shoots in seedlings raised in growth media containing saw dust or sterilized soil. In seedlings that received soilapplied fertilizer, shoots were developing at nearly twice the rate of the shoots (Figure 3).

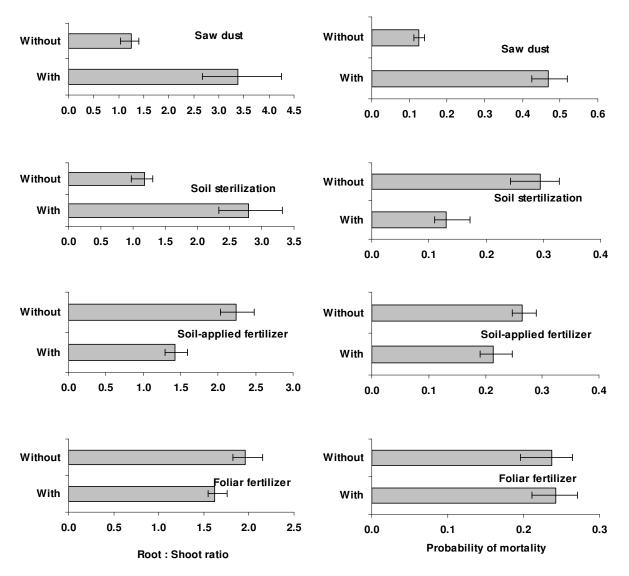


Figure 3. Variation in biomass allocation and seedling mortality with the main effects of treatments. Error bars indicate the 95% confidence intervals.

Table 1. Model selection based on the Bayesian information criterion (BIC) for effect of potting mixture (Mix), soil applied fertilizer (Soil) and foliar fertilizer (Foliar) on *U. kirkiana* seedling height, leaf number, number of primary roots, collar diameter, primary root length, root dry weight, shoot dry weight and root to shoot ratio (RSR).

Model	Height	Number of leaves	Collar diameter	Root length	Root weight	Shoot weight	RSR
Mix Soil Foliar	225	211	293	108	686	685	200
Mix Soil	233	217	312	114	709	736	505
Mix Foliar	232	205	316	118	759	703	537
Mix	238	214	319	129	764	743	612
Soil Foliar	229	223	325	172	830	718	857
Soil	229	218	329	168	839	743	866
Foliar	234	216	327	176	834	720	868

Mix | Soil |Foliar indicates the main and all interaction effects. BIC scores in bold face indicate the best model for each variable.

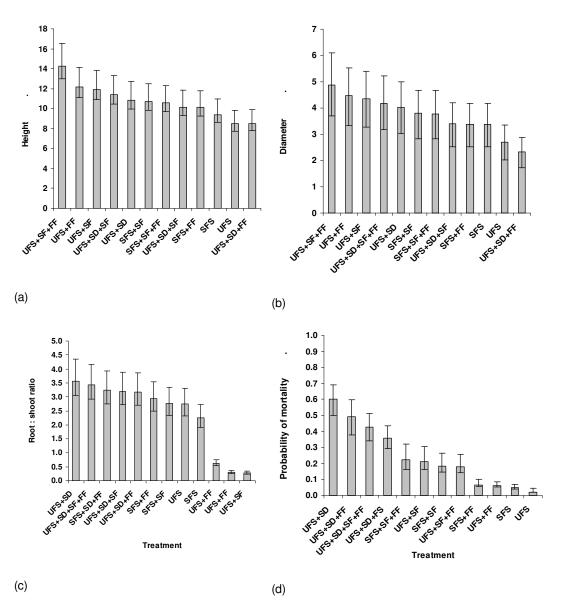


Figure 4. Variation in height (a), root collar diameter (b), biomass allocation (c) and probability of seedling mortality (d) with treatment combinations. UFS = unsterilized forest soil; SD = saw dust; SF = soil applied fertilizer; SFS = sterile forest soil. Vertical error lines indicate 95% confidence intervals.

Foliar disease symptoms including necrosis, leaf spots and blighting were common in seedlings of *U. kirkiana*. The causal agents included the fungal genera *Cercospora*, *Colletotrichum*, *Alternaria* and *Phoma*. The correlation between seedling mortality and foliar disease incidence in October was highly significant (r = -0.948; P<0.0001). The correlation between seedling mortality and number of lateral roots (r = -0.745; P = 0.054) and leaves (r = -0.549; P= 0.065) were negative although marginally significant. Seedling survival was related to biomass allocation in a curvilinear manner (Figure 2). The polynomial regression of foliar leaf disease incidence and seedling mortality on root to shoot ratio explained 77.0 and 64.4% of the variation in disease incidence and seedling mortality, respectively. The probability of plant mortality was higher in the potting mixture where saw dust was added than in the mixture without saw dust (Figure 3). Mortality was also sig-nificantly higher (P<0.05) in unsterilized soil than sterilized soil (Figure 3). Potting mixtures with soil-applied fertilizer had significantly lower (P<0.05) probability of plant mortality compared to those without. Foliar fertilizer application did not significantly influence (P>0.05) the probability of plant mortality (Figure 3). According to the BIC, individually the main effects of soil and foliar application of fertilizer were less consistent in describing plant mortality, but the model that consisted of the main effects and interactions was more parsimonious. The probability of seedling mortality was highest (0.60) in unsterilized forest soil mixed with saw

dust and in the absence of fertilization. Probability of plant mortality was lowest (<0.10) either in unsterilized or sterilized forest soil without sawdust and fertilizer application (Figure 4d).

DISCUSSION

The study revealed differential allocation of biomass to the roots and shoots, which affected growth and survival of plants. Neither potting mixture nor fertilizer application consistently influenced growth, biomass allocation and survival of *U. kirkiana* in the nursery. Seedling height and leaf number which provide a measure of photosynthetic and transpiration area are indicators of seedling quality (Ritchie, 1984) were better in potting mixtures containing unsterilized forest soil without saw dust. Root collar diameter, which is regarded as a measure of general seedling durability and one of the best predictors of field survival (Thompson, 1984), was also better in potting mixtures without saw dust. Along with height and root collar diameter, the height to diameter ratio is thought to be an important stock characteristic which influences early plantation performance in trees (Burdett, 1990). Height to diameter ratio of U. kirkiana seedlings was improved when unsterilized forest soil without sawdust was used. Root to shoot ratio indicates the relative proportion of growth allocated to roots versus shoots in a given condition. In this study, inclusion of saw dust in the growth medium and soil sterilization increased biomass allocation to roots more than shoots, while fertilizer application had the opposite effect. These results indicate that growth and distribution of dry matter in seedlings of U. kirkiana are altered by the inclusion of saw dust in the growth medium, sterilization of the soil or fertilizer application.

Though, the potting mixture widely used in the region (75% forest soil + 25% sawdust) has been reported to be lighter, with higher total porosity, water holding capacity and infiltration than forest soil alone (Swai, 2002; Mhango, 2000), it resulted in unbalances in biomass allocation and poor seedling performance. This potting mixture increased biomass allocation to roots than shoots especially when fertilizer was applied. This is probably because the saw dust had immobilized the nutrients in the soil hence leading to a nutrient stress. Ecological studies indicate that root/shoot allocation in plants is influenced by stress factors (Mooney and Winner, 1991; Reynolds and Thornley, 1982). Tree seedlings growing under water-stress or nutrient-stress are known to show higher root to shoot ratios because plants shift relatively more assimilates to the roots in drought-prone and nutrient-poor environments (Gedroc et al., 1996; Lloret et al., 1999). Shifting growth patterns allow plants to compensate for resource limitations by increasing allocation to organs or functions most closely related to acquisition of the limiting resource (Reynolds and Thornley, 1982; Mooney and Winner, 1991). Increased number of side roots and hence more biomass allocation to roots in the potting mixture containtaining sterilized forest soil is probably a result of elimination of mycorrhizal, which are associated with root development and nutrition of *U. kirkiana* seedlings (Högberg, 1982; Mwamba, 1995). Sterilization of the soil could have killed most of the mycorrhizae fungi hence interfering with uptake of nutrients such as phosphorus. Mycorrizal fungi have mostly been known to improve root shoot ratio and seedling survival and growth by enhancing uptake of nutrients, water and protecting the root system against soil-borne pathogens (Harley and Smith, 1983). The effect of mycorrhiza on the nutrition and biomass allocation of *U. kirkiana* seedlings needs to be further investigated.

Seedling mortality was generally lower in sterilized soil probably because sterilization has eliminated soil-borne diseases. Besides soil sterilization, the interaction effects of fertilizer application and growth media also influenced seedling mortality. Therefore, it is concluded that survival of *U. kirkiana* seedlings in the nursery is a function of disease incidence, plant growth and biomass allocation, which in turn are functions of the growth medium used. This indicates the need for development of an integrated nursery management practice that improves the overall health of the plant rather than efforts aiming at disease control, fertilization and other management practices in an isolated manner.

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