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Aerial and Below Ground Biomass Production of Acacia as Influenced by Organic Waste Substrates During Nursery-Stage Seedling Growth

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Abstract: The influence of growing substrates, primarily organic waste, was determined in a greenhouse experiment on four *Acacia* species. The effect of digested sewage sludge, pine litter, oak litter and perlite on aerial and below ground biomass production was evaluated in 3 substrate volumes. Sewage sludge induced significantly greater biomass production and enhanced growth of *A. aneura*, *A. farnesiana*, *A. saligna* and *A. victoriae* over all other media in all measured parameters. The sewage sludge substrate of 25% quartz sand, 25% silty clay soil and 50% digested, dried sewage sludge (v/v) had higher mineral content and water retention rates than all other media. It was superior in dry biomass production of roots and shoots. Results also indicated inhibited growth in oak (*Quercus coccifera*) litter as plant development progressed. It was concluded that (a) digested sewage sludge can be used as part of the growth substrate to enhance *Acacia* seedling growth, and (b) that organic waste substrates could partly replace more expensive nitrogen sources in nursery-stage *Acacia* production. The physical, chemical and biochemical characteristics of sewage sludge in relation to plant growth are discussed.

Keywords: biomass, *Acacia*, sewage sludge, oak litter

INTRODUCTION

Differences or similarities among plant species in their requirements for successful germination and establishment have important implications for the maintenance of species diversity. The genus *Acacia* contains diverse growth forms including freestanding trees, shrubs and highly tolerant bushes (Gutteridge 1992, Miller and Bayer 2001). These are used as a source of firewood, animal nutrition, building materials and for medicinal purposes (Hall 1972, Pressland 1975, Sheikh 1988, Tiedman and Johnson 1992).

Patterns of germination and establishment can be impacted by the overall characteristics of the different habitats in which *Acacia* grows (Doran et al. 1983, Vellend et al. 2000). However, a common factor that impacts successful establishment is the early growth phase from emergence to the end of the first year of seedling growth (Stout 1935, Oliver 1971, Starr 1983, Al-Mudaris et al. 1998). This stage can be characterised by slow, stagnated growth. Although there is considerable variation in *Acacia* response to nursery-stage practices, in many cases growth is enhanced by the addition of organic material to the growth substrate (Fox and Leeuwen 1985). Given the significance of *Acacia* in afforestation and reforestation efforts in arid and semi-arid savanas in North Africa and the Middle East (Blunt 1926, Whibley 1980, Simmons 1987), it is important to determine the degree to which locally occurring organic substrates could be used to enhance the growth of *Acacia*. The basis for use of organic wastes in plant growth substrates has been an interesting topic for recent studies (Schumann and Sumner 2000, Garling and Boehm 2001).

Comparative studies of varying growth media may be particularly informative because potentially significant growth gains may be made; thus shortening the nursery stage and improving stand establishment, both important factors in *Acacia* production (Simmons 1988).

To examine the interactive effects of local organic waste media, *Acacia* species and substrate volume, we grew *Acacia* seedlings in a split-split block design experiment. Our objectives were to determine whether growth in organic wastes, including sewage sludge and leaf litter, improved above and below ground biomass production relative to conventional nursery media, and whether these effects were modified by species and/or substrate volume. Factors controlling biomass allocation, nutrient impacts on growth and physical substrate characteristics were also evaluated.

MATERIALS AND METHODS

Plant Material

Seeds of *Acacia aneura*, *A. farnesiana*, *A. saligna* and *A. victoriae* were obtained from the Australian Tree Seed Centre in Canberra, Australia. These were treated via acid scarification (sulphuric acid) following Al-Mudaris et al. (1998) (soaked for 45 minutes in H_2SO_4 at room temperature and rinsed in distilled water) and sown into Rootainer$^{®}$ cells (Hummert International, USA) (Maherali and De Lucia 2000). Three-week-old seedlings were randomly plucked and used for this experiment. All seedlings had healthy roots and initial leaf formation.

Measurements were taken from the tip of the root to the tip of the apical meristem, from the crown to the apical meristem and from the crown to the tip of the root. These were taken to be the initial length of above and below ground biomass and the complete plant on initiation of the experiment.

Organic Waste Substrates

The three sources of organic waste chosen to substitute traditional, more expensive peat moss-based growth media were digested sewage sludge, pine forest litter and oak forest litter. In addition, perlite was used as a non-organic, inert substrate to improve air-filled porosity and stability of the media.

Digested, dried sewage sludge was obtained from Al-Salt sewage treatment plant, 7 kilometres to the north of Salt City, Amman, Jordan. The plant is situated at a longitude of 35.73, latitude of 32.03 and altitude of 796 m. It processes 3800 cubic meters of sewage a day. The local area has a population of 57 000, an annual precipitation rate of 557 mm and an average air temperature of 18°C, reaching over 40°C in the hot summer months.

The sewage sludge had been bacteria-digested, spread and dried for 2 months prior to being used in this investigation. Biochemical and nutrient analyses were conducted on sewage samples following Olsen and Dean (1965).

Pine and Oak Litter

A local forest, the Scandinavia, with an 80 to 200 year-old stand of pine (*Pinus halepensis*) and oak (*Quercus coccifera*) was used as the collection site for litter. The forest receives annual precipitation of 500 to 534 mm with slopes of 10–60%. Covering litter and the top 8–10 cm of soil, mostly organic matter, was taken from under the canopy of 40 random trees of pine and oak. A composite sample was obtained by mixing the 40 litter/soil collections, sieving through a 3 mm screen and spreading in a 5-cm thick layer. All samples were air-dried in the sun for 10 days.

Standard Growth Media

Perlite, an inert, commercial substrate, was purchased from a local manufacturer (Dawood, Jordan). Sand and nursery-grade silty clay soil were obtained from a nursery supplier (Al-Ain, Jordan).

Analyses

All media components and mixtures were analysed for chemical and physical characteristics. Nitrogen was analysed using the Kjeldahl method (Bremner 1965), phosphorous, potassium, iron, zinc, copper, cadmium and organic matter were analysed following Olsen and Dean (1965). Bulk density, texture (pipette method) and water retention (ceramic plate method) were also determined (Olsen and Dean 1965).

Polybags

In order to evaluate the impact of substrate volume on *Acacia* biomass production three polybag sizes were used to grow seedlings over the 12-month experimental trial period. These were 12 L, 8.2 L and 6 L. These were made of Grade 1 polythene and purchased from a local stockist (Ahram Plastic, Jordan).

Substrate Components

Four treatments and a control were used to evaluate media impact on growth and biomass production. These were Control, oak litter, pine litter, perlite and digested sewage sludge. The Control was composed of 50% sand and 50% silty clay soil. Oak litter was mixed with sand and silty clay at a ratio of 50:25:25% (volume basis), respectively (hereafter termed Oak). Pine forest litter (Pine), perlite (Perlite) and sewage sludge (Sludge) were mixed with sand and silty clay at the same ratios.

All growth substrates were weighed into polybags and *Acacia* seedlings transferred into them after irrigation with 350 mL water. Thereafter, seedlings were irrigated with 300 mL/week. Samples of the irrigation water were taken monthly and analysed for pH and electrical conductivity (EC). No fertiliser applications were made, and weed management was performed manually as required. Whilst fertilizer application is standard practice in most nursery production facilities today, it is not always the case in local Jordanian seedling production. It should be noted that the lack of fertilizer application was taken to be the worst case scenario of local Jordanian *Acacia* seedling production.

Temperature and relative humidity measurements were taken daily using a Jules, Richard and Pekly® thermohygrograph (JR&P, UK). Growth substrate temperatures were measured twice daily at 7am and 3pm using an electrode thermometer (Hereaus, Germany).

Statistical Analysis

The experiment was performed as a Randomised Complete Block Design (RCBD) with a split-split block arrangement. Each treatment was replicated 5 times for every species and polybag size totalling 300 experimental units. The General Linear Model ANOVA was performed (Barrilleaux and Grace 2000) with mean separation at 5% using Duncan's Multiple Range Test.

Plant Growth Measurement and Biomass Production

Measurements were taken twice a month for 12 months and included plant height (from the crown area to the tip of the apical meristem) and stem diameter. The latter was measured using a Gibbons® gauge (Gibbons, UK) micrometer at 2 cm above the crown region.

At 12 months of age, seedlings were harvested, cleaned and weighed. From these measurements, the fresh mass of shoot (FMS) (representing total aerial biomass) and fresh mass of root (FMR) were obtained. Shoots and roots were separated and dried in an airflow cabinet (Conviron Industries, Canada) at 70°C for 3 days or until sample weight stabilised. Dry samples were weighed and placed in dry storage. These measurements produced the dry mass of root (DMR) and dry mass of shoot (DMS). For brevity, only growth data at 2, 6, 9 and 12 months will be shown in addition to final above and below ground biomass production.

RESULTS AND DISCUSSION

Treatments

Aerial and below ground biomass production revealed significant differences between treatments, indicating substantial growth enhancement or suppression. We found Pine, Perlite and Sludge to induce higher growth just 2 months after initial transfer to growth media over Control- and Oak-grown plants (Table 1). By the end of the 6th month, a further significant difference appeared among these treatments with Sludge-grown plants clearly outperforming Pine and Perlite counterparts by 1.3 times (calculated from Table 1). No significant differences were detected between Control and Oak plants. This pattern of growth continued until month 9 with Sludge significantly outperforming all other treatments. After 12 months of growth, *Acacia* seedlings grown in Sludge reached a height of 116.1 cm followed by 69.5 cm for Pine and 64.6 cm for Perlite. This difference was statistically significant as was the difference between Control (56.5 cm) and Oak (42.7 cm). Sludge plants were 1.6 times higher than Pine and double the height of Control plants.

Stem diameter demonstrated a slightly delayed differentiation in Sludge with significantly higher stem diameter values appearing after 9 months of growth, but not earlier (Table 1). Oak produced shorter and thinner seedlings than even the Control. In fact, at 12 months of age Oak-grown seedlings only had 0.6 the stem diameter of Control and 0.4 that of Sludge. Oak exhibited a clear degree of suppression in seedling growth.

Analysis of biomass production revealed a positive correlation with plant height and diameter, where Sludge produced the highest biomass in terms of FSM, DSM, FRM and DRM (Table 2). There was no variation in the allocation of biomass between shoot and root as shoot: root ratios for all treatments ranged from 1.8 to 1.5 (data not shown). This indicates that treatments did not alter the ratio of allocation of biomass.

Substrate	**2 months**	**6 months**	**9 months**	**12 months**
		Height (cm)		
Control	2.3 b	13.1 c	30.1 c	56.5 d
Oak	3.1 b	13.8 c	26.9 c	42.7 d
Pine	4.1 a	19.3 b	40.8 b	69.5 b
Perlite	4.5 a	19.4 b	38.1 b	64.6 c
Sludge	4.4 a	27.1 a	59.1 a	116.1 a
		Diameter (mm)		
Control	2.2 c	5.9 b	9.1 c	10.6 c
Oak	3.1 bc	4.9 c	6.1 d	7.2 d
Pine	3.8 a	7.3 a	12.1 b	14.0 b
Perlite	3.4 b	6.8 a	10.7 b	13.0 b
Sludge	3.3 bc	7.4 a	13.9 a	17.1 a

Table 1. Effect of growth substrate on stem height and diameter in *Acacia* over a 12-month period under greenhouse conditions.
Mean values within columns are not significantly different at $p \leq 0.05$ if sharing the same letter/s within the same parameter (height or width). Mean separation conducted using Duncan's Multiple Range Test.

Substrate	FSM (gm)	DSM (gm)	FRM (gm)	DSM (gm)
Control	30.6 bc	16.1 bc	16.4 cd	8.9 c
Oak	21.5 b	12.3 c	12.4 d	7.5 c
Pine	39.7 b	21.7 b	22.3 b	13.1 b
Perlite	35.1 b	19.3 bc	19.3 bc	10.7 bc
Sludge	81.8 a	42.5 a	48.1 a	27.8 a

Table 2. Effect of growth substrate on aerial and below ground biomass production in *Acacia* after 12 months of growth.
FSM: Fresh Shoot Mass, DSM: Dry Shoot Mass, FRM: Fresh Root Mass, and DRM: Dry Root Mass. Mean values within columns are not significantly different at $p \leq 0.05$ if sharing the same letter/s. Mean separation conducted using Duncan's Multiple Range Test.

Polybag

Polybag size, and hence substrate volume, only induced significant differences in plant growth, averaged over all treatments, after 6 months of growth. The 12 L polybag showed higher biomass values in terms of plant height, stem diameter, FMS, DMS, FMR and DMR over the other 2 sizes.

Species

Acacia farnesiana exhibited significantly higher growth and biomass production in all media and polybags, followed by *A. saligna*, in comparison to the other 2 species (data not shown). This difference was apparent across the four growth phases at 2 months, 6 months, 9 months and 12 months. *A. aneura* and *A. victoriae* were similar in their growth patterns, each growing to 0.8 the height and stem width of *A. farnesiana*. The same applied to FMS, DMS, FMR and DMR over all four growth phases.

Interactions

Interactive analyses revealed similar results (data not shown) where Sludge out-performed all other treatments and no preference for a particular species or polybag existed. Similarly, all species responded favourably to Sludge and large polybags, and all polybag sizes responded favourably to Sludge. Oak clearly inhibited growth in all species and polybag sizes. The highest overall biomass allocation in all measured parameters was attained in *A. farnesiana* grown in Sludge in 12 L polybags.

The most notable result of these experiments was the positive aerial and below ground biomass allocation in Sludge-grown plants. These results were evidenced by substantial increases in seedling mass, stem growth and root growth in plants exposed to Sludge treatment.

Digested, dried sewage sludge/organic waste has been shown to release nitrogen and phosphorous at rates significantly impacting plant growth (Williams and Whitcombe 1988, Eghball 2000). Indeed, in this study, chemical analysis revealed substantially higher N content in Sludge compared to other treatments. Specifically, Sludge had 1.5 and 3.5 times higher N content than Pine and Oak, respectively, and 4 times more N content than Control and Perlite. The same applied to P, Fe and Zn. As an example, whilst Control had a P content of 3.3 ppm, Sludge had 49.7 ppm. Sludge also had 39.0 ppm Fe compared to 0.9 ppm in Control. Overall, Oak, Pine and Sludge had higher organic matter (5.9, 6.0 and 7.2%, respectively) content than Control or Perlite.

Bulk density was similar for all treatments ranging from $1.4\,\mathrm{g\,cm^{-3}}$ for Control to $1.06\,\mathrm{g\,cm^{-3}}$ for Perlite. However, an interesting difference was observed in pH and EC levels of Sludge compared to other treatments. Whilst the pH for all other treatments averaged 8, that of Sludge was 6.9. It was more acidic than the other treatments. Sludge also had higher salt content as it reached 1.90 dS m^{-1} compared to an average of 0.40 dS m^{-1} for all other treatments. This points to an altered mineral balance in Sludge with higher N content being balanced by high Na, Cl and Mg content (Kader, unpublished data), which has been shown to affect growth (Glenn and Brown 1998, Houle et al. 2001). Irrigation water pH and EC over 1 year averaged 6.7 and 0.8 dS m^{-1}, respectively.

The higher salt content may reach levels high enough to induce stress (Williams et al. 1998) or alter physiological responses (Howard and Mendelssohn 1999) and growth (Meiners et al. 2002). In this case it appears that Sludge mineral content was sufficient to cause improved growth without any toxicity damage as reported in previous studies for other species (Glenn and Brown 1998).

An additional interesting characteristic of Sludge was its high moisture retention capacity at high-tension levels (lower moisture). At 0.1 bar, Control, Pine, Perlite and Oak averaged a moisture content of 41% (gravimetric) compared to 46% for Sludge. As tension increased to 0.5 bar, this dropped to 29 and 39%, respectively; and to 9.5 and 20%, respectively at 5.0 bar. The largest difference was observed at 10 bar (the permanent wilting point of plants is 15 bar). Here, sludge retained 15% of the moisture content compared to an average of 4% for all other media. This would have a large impact on the solubility of nutrients and movement up the plant roots, thus impacting growth, regeneration (Swagel et al. 1997) and root distribution (Clark et al. 1999).

We observed higher average temperatures in Sludge over all other media across all months of the year and all growth phases. Despite the fact that the sludge used was digested, it revealed significantly higher microbial activity and respiration rates over all other media (Kader et al. unpublished data). The average temperature 8 cm inside the growth medium was 21.2°C across all substrates (excluding Sludge), months, species and polybags. The comparable temperature of Sludge was 26.5°C. Higher substrate temperatures have been found to induce greater nutrient mobilisation rates (Wang and Roberts 1983), higher sink effectiveness and higher associated growth rates (Bailey and Jones 1941) and substrate nutrient release (Giardina et al. 2000). Other changes in substrate temperature and composition may have an impact on phosphorus (Garcia-Montiel et al. 2000), but seedlings may adapt to these situations (Williams et al. 1998, Boorse et al. 1998).

Oak, as a medium, did not induce *Acacia* growth despite its high organic matter content, nitrogen level and good water retention characteristics. This is in line with previous work in this laboratory, which may suggest an inhibitory effect of *Quercus* on other plant species (Kader et al. unpublished data, von Renesse, personal communication) despite the physical enhancement of substrate characteristics (Niklas 1999).

Polyphenols, terpenes and allelopathy have been suggested as causal factors behind reduced growth (Karabourniotis et al. 1998, Llusia and Penuelas 2000, Gordon and Rice 2000). Recent research has shown effects ranging from reduced growth (Kamara, 1998) to inhibition of seedling survival (Nilsen et al. 1999) through chemical (Rice 1979) and other (Gopal and Goel 1993) factors in oak (Abrams 1992).

One of the potential limitations of sewage sludge is the possible toxic effect from high levels of heavy metals it is associated with. Analyses in this laboratory have shown Fe, Zn, Cu and Cd levels of 300, 42, 79 and 37 ppm, respectively. These are sufficiently high enough to cause toxicity to plants and accumulation in plant parts (Hinesly et al. 1978) and has been identified as a health risk, especially in plants of an annual nature (Jensen and Lesperance, 1971). However, there appears to be limited risk associated with plants grown in sewage sludge at the nursery stage, where there is limited likelihood of grazing. *Acacia* seedlings also accumulate less Fe, Zn, Cu and Cd with progressive growth, as analyses in this experiment (Omari et al. unpublished data, Hattar et al. unpublished data) and other time-dependent studies (Wallis et al. 1984, O'Conor 1991) have shown.

A more pressing constraint on sludge utilisation would appear to be the direct health risk associated with the microbiological content of sludge. The sludge used in this experiment contained 10^8 colony forming units (CFU) g^{-1}. It contained both aerobic and anaerobic bacteria in addition to a positive mycological reading. This is in line with previous work (Hamparian et al. 1985) and points to a need for well-defined nursery operations management to accompany such utilisation. The present high microbiological activity would have increased respiration rates inside the substrate and consequently raised it's temperature leading to the observed temperature measurements in Sludge compared to the other substrates. It is of relevance to point out that in New South Wales (Australia), there is a set of guidelines that control the use and application of biosolids issued by the NSW Environmental Protection Authority. These impact the application of sewage sludge in agricultural, horticultural and other areas and may be referred to for guidelines (EPA, 1997).

The fact that *Acacia* growth was significantly higher in 12 L polybags compared to the smaller sizes may be attributed to the need for larger root growth space from 6 months onwards. Samples of bags cut open at this stage revealed a tighter spiralling of roots in the smaller sized bags compared to a better-distributed root system in large bags. This appeared to be the case for all species in all growth media, but was most pronounced in Sludge, where root growth proceeded at a higher rate. A slight departure from this trend was observed in Perlite where roots penetrated the Perlite particle and appeared to circumvent it, creating knot-like root systems.

Perlite is derived from siliceous volcanic rock. It contains some 2–5% moisture and, when crushed and heated to about 1000°C, expands to form a lightweight perlite particle with a closed cellular structure (Verdonck 1983). It is chemically inert and serves as a substrate aeration medium, which enhances root growth in heavier substrates (Paul and Lee 1976). Earlier reports have shown enhanced nitrate, soluble phosphorous, potassium and magnesium uptake due to this aeration (Shanks and Laurie 1949). The aerating effect was also achieved in Pine, as it's bulk density was 0.7 that of the Control. It also had 3.2 times more organic matter. This effect has been found to enhance root elongation and nitrogen intake (Batier et al. 1943, Huck 1970) as well as higher availability of nutrients, due to leaching from tree litter in the substrate.

Based on the results of the present study, digested sewage sludge mixed with sand and soil at a ratio of 25:25:50% (v/v) appears to enhance aerial and below ground biomass production associated with faster growth in *Acacia* seedlings. It is notable that under Australian nursery practices, soil is no longer used in potting mixes, but continues to be used in Jordanian nurseries. Although the results revealed high levels of heavy metal and microbial content in sewage sludge, they suggest higher substrate temperatures and mineral content enhanced early seedling growth in *A. aneura*, *A. farnesiana*, *A. saligna* and *A. victoriae.* Based on the inhibited growth in Pine (*Quercus coccifera*) litter, it is proposed that this medium not be used in potting substrates in *Acacia.* Aeration by way of using Perlite in the growth medium enhances *Acacia* growth.

CONCLUSIONS

It is concluded that sewage sludge in dried, powder format may be used as an alternative potting substrate for *Acacia* seedlings. It produced enhanced aerial and below ground biomass for all *Acacia* species studied and may act as an organic compound of significant practical value in shortening nursery growth periods.

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