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SELECTION AND PROPAGATION OF ELITE *ATRIPLEX* MATERIAL

by

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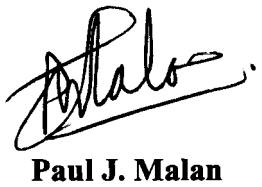
DECLARATION

I declare that this is entirely my own work and has not previously been submitted for any qualification. Where material from other sources has been used it has been referenced in full.

Signed: 

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SUMMARY

The aims of this study were to compare 16 *Atriplex* accessions as fodder shrubs; to find a method of vegetative propagation for *A. nummularia* and *A. canescens*; and to find a non destructive way of monitoring dry matter (DM) production and structure. Characteristics evaluated included DM yield, structure, nutritive value and adaptation to different ecological conditions. The accessions were also compared for palatability using grazing sheep in a cafeteria system. Two methods of vegetative propagation were evaluated, namely *in vitro* tissue culture propagation (*A. canescens* and *A. nummularia*) and stem cuttings (*A. nummularia*).

With all accessions the percentage leaves decreased and percentage twigs increased as the plants matured. Leaves were more nutritious than edible twigs. At an age of 15 months edible DM yield ranged from 0.71 $t.ha^{-1}$ (*A. canescens* Rincon) to 5.78 $t.ha^{-1}$ (*A. rhagadioides*). For most accessions crude protein (CP) content, neutral detergent fibre (NDF), *in vitro* DM digestibility (IVDMD) and ash contents were acceptable for livestock. The two most promising accessions were *A. nummularia* (CP content 15%, NDF 48%, IVDMD 60% and ash 20%) and *A. canescens* cultivar Santa Rita (CP content 13%, NDF 49%, IVDMD 58% and ash 18%). The two least promising accessions as fodder shrubs were *A.glauca* (CP content 8%, NDF 61%, IVDMD 42% and ash 16%) and *A. canescens* Rincon (CP content 10%, NDF 58%, IVDMD 44% and ash 15%).

Survival at different locations were not only influenced by ecological and climatological conditions, but also by different management practices. Average survival ranged from 41% to 87%. Sampling for nutritive value varied between locations and had a stronger influence on the nutritive values than location. Accessions with the broadest adaption were *A. amnicola, A. canescens* (S.R.), *A. halimus* and *A. nummularia*.

According to visual observation and utilization, *A. amnicola*, *A. canescens* (S.R.), *A. cinerea*, *A. rhagadioides* and *A. semibaccata* were the most palatable accessions. The sheep, contrary to expectations, were able to select equally nutritious diets over time.

In vitro propagation of *A. nummularia* was more successful than *A. canescens*. The best medium for shoot multiplication and elongation for *A. canescens* was the Murashige and Skoog (MS) basal medium with 0.20 $mg.l^{-1}$ 6-benzylaminopurine (BA) and 1.0 $mg.l^{-1}$ naphthalene acetic acid (NAA), while a medium with 0.05 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA was best for elongation and rooting. For multiplication of *A. nummularia* the MS basal medium with 0.20 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA was the best, while the MS basal medium without any hormones was the best for elongation and rooting. *Atriplex nummularia* was successfully acclimatized.

Vegetative propagation using stem cuttings was also possible for *A. nummularia*. The best results (up to 95% rooting) were obtained using terminal cuttings of new growth in the autumn or spring, treated with 3 $g.kg^{-1}$ indole-3 buteric acid (IBA).

Suitable models for the prediction of DM yield, using non-destructive measurements were found. The model: $DM = 553.24X + 129.47$, where, DM = dry matter yield ($g.plant^{-1}$) and X = shrub volume (m^3), gave very good predictions ($r^2 = 0.72$; $P = 0.0001$). Other models, which were species specific, included: DM yield in relation to volume; height and diameter in relation to DM yield; and shrub volume related to DM yield, where each accession has its own intercept while, the slope were the same for all. Acceptable models were found for all accessions with the exception of *A. semibaccata*. Another model should be tested for this accession with its creeping growth form.

OPSOMMING

Die doelwitte van hierdie studie was om 16 *Atriplex* tipes as weigewasse te vergelyk; om 'n vegetatiewe metode van vermeerdering vir *A. nummularia* en *A. canescens* te ontwikkel; en om 'n nie vernietegende metode om droë material (DM) produksie en struktuur te skat, te kry. Eienskappe wat evalueer is het DM produksie, struktuur, voedingswaarde en aanpassing by verskillende ekologiese toestande ingesluit. Die smaaklikheid van die *Atriplex* tipes was ook deur middel van skape, in 'n kafeteria sisteem bepaal. Twee metodes vir vegetatiewe vermeerdering was bestudeer, naamlik: *in vitro* weefselkultuur vermeerdering (*A. canescens* en *A. nummularia*) en vermeerdering met steggies (*A. nummularia*).

Die persentasie blare het afgeneem en die persentasie stingels van alle tipes het toegeneem namate plante ouer geword het. Blare het 'n hoër voedingswaarde as vreetbare stingels gehad. Op 'n ouderdom van 15 maande het vreetbare DM opbrengs gewissel van 0.71 $t.ha^{-1}$ (*A. canescens* Rincon) tot 5.78 $t.ha^{-1}$ (*A. rhagadioides*). Meeste *Atriplex* tipes was, op grond van die ru-proteïen (RP) inhoud, neutraal bestande vesel (NBV), *in vitro* DM verteerbaarheid (IVDMV) en as inhoud, aanvaarbaar vir vee. Die twee belowendste tipes was *A. nummularia* (RP inhoud 15%, NBV 48%, IVDMV 60% en as 20%) en *A. canescens* Santa Rita (RP inhoud 13%, NBV 49%, IVDMV 58% en as 18%). Die twee tipes wat die minste geskik was as voergewasse was *A.glauca* (RP inhoud 8%, NBV 61%, IVDMV 42% en as 16%) en *A. canescens* Rincon (RP inhoud 10%, NBV 58%, IVDMV 44% en as 15%).

Oorlewing van *Atriplex* tipes by die verskillende lokaliteite was deur ekologiese-, klimatologiese- en bestuurstoestande beïnvloed. Gemiddelde oorlewing het van 41% tot 87% gewissel. Monsterneming het tussen lokaliteite verskil en het 'n groter invloed op voedingswaarde gehad as die lokaliteit. Die *Atriplex* tipes wat die breedste aanpassing getoon het, was *A. amnicola, A. canescens* (S.R.), *A. halimus* en *A. nummularia*.

Volgens visuele waarneming en benutting deur skape was: *A. amnicola*, *A. canescens* (S.R.), *A. cinerea*, *A. rhagadioides* en *A. semibaccata* die smaaklikste tipes. Anders as verwag kon die skape oor tyd, diëte hoog in voedingswaarde, selekteer.

In vitro vermeerdering van *A. nummularia* was meer suksesvol as vir *A. canescens*. Die beste medium vir stingelvermeerdering en -verlenging van *A. canescens*, was die Murashige en Skoog (MS) basiese medium met 0.20 $mg.l^{-1}$ 6-benziel aminopurien (BA) en 1.0 $mg.l^{-1}$ fenielasynsuur (PAA), terwyl 'n medium met 0.05 $mg.l^{-1}$ BA en 0.05 $mg.l^{-1}$ PAA geskik was vir stingelverlenging en wortelvorming. Vir vermeerdering van *A. nummularia* was die MS basiese medium met 0.20 $mg.l^{-1}$ BA en 0.05 $mg.l^{-1}$ PAA die beste, terwyl die MS basiese medium sonder enige plant hormone die beste vir stingelverlenging en wortelvorming was. Akklimatisering van *A. nummularia* was suksesvol.

Vegetatiewe vermeerdeing van *A. nummularia* met steggies was moontlik. Die beste resultate (tot 95% beworteling) was verkry deur van terminale steggies van nuwe groei in die herfs of lente, wat met 3 $g.kg^{-1}$ indool-3 bottersuur (IBA) behandel is, gebruik te maak.

Geskikte modelle vir die voorspelling van DM opbrengste is gevind. Die model: DM = 553.24X + 129.47 waar, DM = droë materiaal opbrengs ($g.plant^{-1}$) en X = struik volume (m^3) het goeie resultate ($r^2 = 0.72$; $P = 0.0001$) gelewer. Ander modelle, wat spesiespesifiek was, soos die verwantskappe tussen DM opbrengs en volume; hoogte en deursnee; en struik volume waar elke tipe sy eie afsnit het terwyl die helling vir almal dieslefde is, is ook getoets. Aanvaarbare modelle is vir alle tipes gevind met die uitsondering van *A. semibaccata*. 'n Ander model sal vir hierdie tipe, met sy kruipende groeivorm, ontwikkel moet word.

CHAPTER I

INTRODUCTION - SURVEY OF LITERATURE

1. Introduction

The genus *Atriplex* belongs to the Chenopodiaceae family and consists of 417 species (Osmond, Björkman & Anderson, 1980). The *Atriplex* genus is the largest and most diverse genus in this family. Most of the species are perennial and woody although there are a few herbaceous annuals. The majority of the species are halophytes, some are xerophytes and a few are ruderals. About 50 species are recognized as fodder shrubs, of which 15 are cultivated as fodder crops (Sankary & Goodin, 1985; Ormazabal, 1991; König, 1992).

Atriplex spp. are found throughout the world but are more specific in semi-arid and arid areas where they are adapted to a wide range of alkaline and saline soils (Watson, 1990; Ormazabal, 1991). According to Aronson (1985) *Atriplex* spp. do not only survive but also produce exceptionally large quantities of biomass under extremely saline conditions. From there the common name of saltbushes. Other important characteristics are tolerance to saline water, adaptation to high and low temperatures and drought resistance (Ansley & Abernethy, 1985; König, 1992). According to Senock, Borrow, Gibbens & Herbel (1991), the wide range of morphological and ecotypical variation within *Atriplex canescens* (Pursh), expresses the wide adaptation within the genus. This adaptation to a wide range of environmental conditions makes the cultivation of *Atriplex* spp. on marginal land possible.

One of the most important characteristics of this genus, is its potential as fodder shrub in arid and semi-arid areas. Characteristics which make these species promising fodder shrubs are the fact that they are perennial, evergreen (even in the dry season), have a high production and have a high nutritive value (de Kock, 1967; Mikhiel, Meyer & Pendleton, 1992). Attempts to utilise these valuable plants throughout the world, have met with mixed results. Reasons for this include a lack of knowledge on germination requirements, methods of establishment, methods of utilization, genetic variation within species and microbiological characteristics (Jacobs & Smit, 1979; Watson, 1990; Mikhiel *et al*, 1992).

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2. Morphology and physiology

The genus *Atriplex* consists of evergreen, herbaceous to woody, shrubby plants most of which are perennial (Osmond *et al*, 1980; König, 1992). A few short lived perennial species are found in Australia, of which *A. holocarpa* F. Muell, *A. angulata* Benth. and *A. lindleyi* (F. Muell) P.G. Wilson, are examples. Annual species such as *A. persistens* Stutz & Chu and *A. rosea* L. are also found in California (Watson, 1990; Stutz & Chu, 1993). Most of the species are upright growing, with heights ranging from 0.5 to 5.0 metre. Prostrate (*A. undulata* Dietr.) and creeping (*A. semibaccata* R. Br.) forms are also found (Abdul-Halim, Al-badri, Yasin & Ali, 1990; Watson, 1990). Some of the annuals are herbs, only a few centimetres in height.

The leaves are scurf with bladderlike hairs (trichomes) on the surface, light green to grey, elongated to oval and 1 - 3 centimetres (cm.) in length (König, 1992). Flowers are carried terminally in clusters or spikes and wind pollination takes place in most of the species. The fruit consists of a single seed, encased in two bracteoles . Almost all species are dioecious - male and female flowers are carried on different plants. A few monoecious species occur amongst the annual species and also in the tetraploid form of *A. canescens* (Zoharry, 1966; Barrow, 1987). According to Strawbridge, Bell, Mc Comb & Barrett-Lennard (1997), up to 40% of plants in an *Atriplex* population can alter their sexual phenotype. Changes of sexual phenotype have been influenced by environmental conditions such as drought, irrigation and extreme temperatures and also as the plants mature. This sexual lability may influence the male to female ratio in a plantation, set up for optimum seed production.

The tap-root can reach a depth of four metres (m.) and lateral roots can be found up to 10 m. away from the plant. Many of the species, of which *A. undulata* is one example, tend to form new roots where side branches touch the soil (Watson & O'Leary, 1993).

Most of the *Atriplex* spp. follows the C_4- photosynthetic pathway. The C_4-pathway is normally an advantage in areas with high light intensities and high temperatures. The advantage of the C_4-pathway is the enhancement of plant growth in the absence of photo respiration (Osmond *et al*, 1980).

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2.1 Halophytic adaptation

Atriplex spp., as halophytes, possess the ability to develop normally under extremely high levels of salinity. This is a genetic phenomenon which separates them from most conventional crops (Glenn & O'Leary, 1984). The adaptation of these plants to a habitat with a low water potential and saline conditions, always results to the accumulation of organic ions, normally in the plants vacuoles (Koheil, Hilal, El-Alfy & Leistner, 1992). These ions will be transported to and stored in older parts of the plant to enhance normal growth and development of young shoots and roots. Salts are transported to the trichomes in the epidermis of the leaves, from where they are excreted. According to Taha, O'mar & Nasset (1990), *Atriplex*'s success as halophytes arises from their ability to store Na^+ and to balance it by the synthesis of an oxalate anion in the leaves. As soon as the salt concentration in the trichomes reaches a toxic level, the trichomes will burst open and excess salt be excreted.

To regulate the osmotic potential within plant cells, quaternary ammonium compounds, for example betaine, are produced in the cytoplasm to neutralise the effect of the organic compounds in the vacuoles. Another advantage is that betaine protects enzyme activity from the deleterious effects of the high salt concentration (Koheil *et al*, 1992).

Such quaternary ammonium compounds might be the reason for the high nitrogen (N) concentrations in *Atriplex* species. Soluble N, which can not be utilized effectively by ruminants, increases with a slight increase in salt content of irrigation water. According to Nerd & Pasternak (1992) this increase is not correlated with that of betaine. Koheil *et al* (1992) found that it is not only the salt content of the soil which causes in increase in betaine, but also maturity, climate, water availability and photo period. Changes in the levels of N-rich compounds can only be seen as components of the tolerance mechanism.

Atriplex lampa is adapted to drought conditions and N-poor soils. Trione & Passera (1993) have found that low water availability, reduced growth of all plant organs, but did not affect the absolute N content, both free and bound nitrogen. Despite the salt concentration in irrigation water the absolute N content stayed the same. *Atriplex lampa* developed the following characteristic to adapt to infertile soil in arid areas:

- a low growth rate to compensate for low nutrient and low water supply
- increased N-absorption per unit root mass with a lower energy cost opposed to produce additional roots; and

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- effective inherent N-balance which originates from an effective redistribution within the plant. This might also be true for most of the *Atriplex* species.

2.2 Rhizobium and Mycorrhizae

Nitrogen fixation, associated with roots of non-legumes, is known as one of the most significant components of the N-cycle in a range of environmental conditions, including various extreme environments. Various non-legumes and a few tropical grasses were identified as plants that harbor N-fixing bacteria in and around their roots. Nitrogen fixation in the rhizosphere showed a significant contribution to N-fixation by plants growing in infertile soil under stress conditions (Bilal, Rasal, Mahmood & Malik, 1990). According to Bilal *et al* (1990), *Atriplex* spp. growing on sodic soils, show a high nitrogenase activity. *Atriplex lentiformis* (Torr.) Wats and *A. amnicola* have an exceptional high salt tolerance and give a high biomass production under stress conditions. Nitrogenase activity was higher in the wet season than in the dry season. Most of the diazotrophs (bacterial spores) associated with *A. amnicola* roots were located on the root surface, followed by the rhizosphere. Large amounts of the bacteria were also counted inside the root tissue, which indicated that the bacteria penetrated the tissue. *Enterobacter agglomerans* were the bacteria most commonly found in the soil, with *Azospirillum* spp. the most on the roots. *Azospirillum* spp. are also associated with the roots of other C_4-plants, such as some tropical grasses. Malik, Bilal, Rasul, Mahmood & Sajjad (1991) discovered the following N_2-fixing bacteria in association with roots of *A. amnicola* and *A. lentiformis*: *Enterobacter agglomerans*, *E. cloace*, *E. intermedium* and *Klebsiella pneumoniae*. They also found low levels of delta ^{15}N in shoot tips, which is a phenomenon of N-fixation through microorganisms and not of N taken up by the roots from the soil. Trione and Passera (1993) also mention the occurrence of microbial association in the root zones of *Atriplex lampa* (Gill. Ex Moq) plants.

Most forage plants of arid and semi-arid rangelands are mycorrhizal. The mycorrhizal fungi absorb nutrients (phosphorus being the most important) from the soil and translocate them to the host plant. The mycorrhizal mycelia thus serve as a highly efficient extension of the root systems. According to Trappe (1981) the following *Atriplex* spp. were found to be mycorrhizal: *A. canescens*, *A. confertifolia* and *A. verrucifera*. In several cases, for example, *A. canescens*, some samples were mycorrhizal and others non-mycorrhizal.

The possibility of the occurrence of root associated organisms in the root zones of *Atriplex* spp.

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might explain their survival and high production under sub-optimal soil conditions. According to Osmond *et al* (1980), *Atriplex* spp. are particularly effective in the absorption and utilization of NO_3^- and PO_4^{3-}. It was found that *A. nummularia* and *A. vesicaria* did not respond to nitrogen and phosphorus fertilisation, although the N and P levels within the plants did increase. Processes which interact with development and growth are poorly understood, but appear to be the key to efficient nutrient utilization in these species.

2.3 Genetic variation within species

The fact that there are cultivars and subspecies within some of the *Atriplex* spp. indicates the genetic variation within species. Such examples are: *Atriplex barclayana* subspecies *barclayana*, *Atriplex patula* variation *histata* and *Atriplex canescens* cultivar Santa Rita (Glenn& O'Leary, 1984; Munda, Lambert & Garrison, 1990; Watson, 1990).

Senock *et al* (1991) refer to the wide range of morphological and ecotipical variation within *A. canescens*. Polyploid genotypes contribute to the successful establishment of a plant species in the range of environmental conditions that exist across its geographical range. Populations of *A. canescens*, throughout its range, have been identified according to chromosome number. These include the diploid (2n = 18), tetraploid (2n = 36) and hexaploid (2n = 54). Tetraploids are the most widely distributed.

Mikhiel *et al* (1992) stress the importance of knowledge of variation within species, before comparing species. Watson (1990) also indicates that productivity and nutritive value for a specific environment, will not only be determined by *Atriplex* spp. but also by subspecies or cultivars.

A study by Watson & O'Leary (1993) of the nutrient composition of different *Atriplex* spp. showed differences between species, within species and between seasons. Kessler (1990) also found differences in crude protein and digestibility between different *A. nummularia* plants. In this case some plants were selected above others and this was attributed to unknown chemical characteristics. Jacobs & Smit (1977) also found palatability differences during a grazing trial between different plants of the same species of: *A. nummularia*, *A. lentiformis*, *A. canescens* and *A. breweri*, with the largest differences in *A. nummularia*. They compared palatable with less palatable plants and found differences in chloride, potassium and phosphorus contents and in the ratio between leaf area and leaf mass. In Syria over 15 markedly different ecotypes of *A.*

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nummularia were collected from range improvement areas (Ibrahim, 1981).

According to Ormazabal (1991), differences in palatability might be caused by genetic variation and by: management practices, soil types, rainfall, temperature, planting density, competition and stocking rate.

Sankary & Goodin (1985) stress the importance of hybrid seed production of elite *Atriplex* ecotypes. From the above examples it is clear that a need exists for the breeding of ecotypes within *Atriplex* spp. with the specific characteristics as a fodder shrub.

3. Establishment

3.1 Seed: production, quality and germination.

Seeds of *Atriplex* spp. are small and flat with a circle like form. Average size varies from one to two millimetres (mm.). Seeds are enclosed in two, paper to leather like bracts or bracteoles with various shapes. Size varies from two to 15 mm. The seed together with the two bracts forms the fruit. Each fruit bears only one seed (Vlahos, Nicholas & Malcolm, 1991). Usually the whole fruit is planted. The fruit size and structure is a poor indicator of seed quality, as the seed can not be seen. Not all fruits will contain normal seed, while other fruit will still be immature and need some time to mature.

According to Watson (1990), *A. cinerea* produces seed all year round, while *A. undulata* starts to produce seed two years after planting. Within the specie *A. canescens*, different ecotypes produce different quantities of seed under different environmental condition, (Barrow, 1987). Because of the dioecious nature of *Atriplex* spp. a sufficient ratio of male to female plants are needed in a seed production plot. Male and female plants should be produced by cuttings and then planted in the appropriate ratio. Experiments by Strawbridge *et al* (1997) showed that a population of 1:1 male and female plants of *A. amnicola*, gave maximum fruit fill. Increasing the percentage of females from 50 to 90% increased total seed production but decreased fruit quality. For seed production of *A. canescens* a ratio of 1:4 male to female plants are suggested by Watson (1990).

Seed germination of *A. amnicola* and *A. undulata* varied from 0 to 75% between different seed sources (Vlahos *et al*, 1991). According to Mikhiel *et al* (1992) different germination control

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mechanisms were observed in different *Atriplex* species. Complex dormancy mechanisms might influence artificial germination of *Atriplex* spp. as knowledge on its functioning is limited. The possibility exists that different ecotypes might have different germination percentages, but environmental conditions will also have an effect on dormancy and germination requirements. Seed of some *Atriplex* spp. is long-lived when stored dry and the viability of the seed tends to increase over time. However, according to Silcock, Williams & Smith (1990) chenopods world-wide seem to have short-lived seed plus inhibitors in the surrounding bracts. Seed of some *Atriplex* spp. reached maximum germination, 12 months after harvesting and will germinate until an age of five years with zero germination there after.

The fruit bracts of the fruits contain substances which inhibit germination (Cambell & Matthewson, 1992). According to Osmond *et al* (1980) the fruit bracts of *Atriplex* spp. contains high levels of NaCl (salt) which need to be leached before germination can occur. Seeds of *A. nummularia*, where the bracts were partially removed, had a germination of 51%, while water treated intact fruits had a germination of 75%. In the water treatment, fruits were soaked in water for 48 hours. Water was replaced every 12 hours and in this way the undesirable substances were leached from the fruit bracts (Cambell & Matthewson, 1992). According to Khan & Rizvi (1994) optimum germination for *A. griffithii* var. *stocksii*, was obtained with distilled water and night and day temperatures of 10°C and 25°C respectively.

3.2 Establishment methods

The two most common methods of establishing *Atriplex* spp. is by direct sowing of seed, or by producing seedlings from seed or cuttings under greenhouse conditions (Weisner & Johnson, 1977).

3.2.1 Seedling production

Because of the difficulties in germinating *Atriplex* seed, sowing of fruits in seedbeds, tins, plastic bags or trays before planting is necessary for species like *A. nummularia* (Donaldson, 1990). As soon as seedlings are big enough, they can be hardened off and planted in the field. Abdul *et al* (1990) found the following seedling survival after transplanting of seedlings to a saline soil with a high clay content: *A. lentiformis* 100%, *A. undulata* 72%, *A. semibaccata* 69%, *A. paludosa* 67%, *A. canescens* 50%, *A. amnicola* 44%, *A. halimus* 36% and *A. nummularia* 19%.

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The other method of producing plants is by cuttings. One advantage of this method is the production of a homogenous stand which is good for experimental purposes, and plants with specific useful characteristic can be multiplied (Weisner & Johnson, 1977). According to Koller, Tadmor & Hillel (1958), it is not easy to make cuttings of *A. halimus*, while Weisner & Johnson (1977) experienced 90% success in the production of plants from cuttings of *A. canescens*.

3.2.2 Direct sowing

The direct sowing method is a more practical and economical method to plant large areas with *Atriplex*. One reason why establishment using this method is difficult is the fact that large quantities of seed are needed and seed is usually not readily available (Watson, 1990).

Atriplex canescens is probably the most widely seeded species. Seeds are sown on a well prepared fine seedbed and harrowed with a spike-harrow to ensure good contact between seed and soil. Under low soil moisture conditions, germination and seedling development is poor, but under favourable conditions, good results can be expected (Schweitzer, Bryant & Wester, 1993). According to Watson (1990) *A. lentiformis* and *A. polycarpa* can also be directly sown, at a depth of 0.5 to 1.0 cm. Seed of *A. amnicola*, *A. lentifomis*, *A. paludosa* and *A. undulata* can be sown mechanically, at a spacing of 3 m. in the row and 1.6 m. between the rows, which give a stand of 2000 plants per hectare. Knowledge of the seed quality and germination potential is, however, necessary (Vlahos *et al*, 1991).

According to Vallance (1989), a company in New South Wales was selling mixtures of *A. amnicola*, *A. lentiformis*, *A. nummularia*, *A. undulata* and *Kochia brevifolis* (Australian blue bush), to be sown mechanically. Donaldson (1990) also mentions the possibility of direct sowing of *A. nummularia*, but only under perfect condition with a well prepared, weed free seedbed, good rains and favourable air temperatures with a maximum of 20°C and minimum of 15°C. Under harsh climatic variables in semi-arid areas it is not possible. According to Le Houérou (1993) *A. glauca* can be sown in arid areas with good results.

In natural rangeland, spontaneous establishment of *A. vesicaria* was noted by Eldridge (1990). Abdul *et al* (1990) and Watson (1990), noticed young seedlings of *A. halimus*, *A. lentiformis*, *A. semibaccata*, *A. nummularia* and *A. undulata* in experimental plots. From this it is clear that some of the *Atriplex* spp. might multiply from seed within plantations.

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3.3 Economic aspects of establishment

In Chile the current nett values of *A. nummularia* and *A. lampa* were examined by Ormazabal (1991). It was found that it was not profitable for a landowner to establish *Atriplex* plantations without subsidy from the government. In Table 1 the costs for establishing *Atriplex*, at different densities, in 1991 can be seen.

Table 1 Costs for the establishment of *Atriplex* plants at different densities in 1991 (Ormazabal, 1991).

Density (Plants.ha^{-1})	Density (mxm)	Cost (R.ha^{-1})
625	4.0x4.0	375
830	3.0x4.0	424
1100	3.0x3.0	487
1250	3.0x2.5	532
1600	2.5x2.5	609

According to Colomer & Passera (1990) the input costs for the establishment of *Atriplex* spp. are very high. The plantation must be used for three to four years before the input costs are covered. It has been suggested that it is not economical to plant *Atriplex* spp. purely as a fodder crop, but rather as a multi-purpose crop used as fuel wood, for erosion control and reclamation and utilization of mining soils. According to Wills, Begg & Brosnan (1990) it is economically viable to plant *Atriplex* spp. on soils that are not suitable for other crops. *Atriplex* spp. can also be used profitably in combination with other crops in arid and semi-arid areas.

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4. Different uses of *Atriplex* species

4.1 General

Plants from the genus *Atriplex* have multiple uses. These include: fodder (especially during the dry season), shade and shelter for livestock, windbreaks, control of runoff water, erosion control, utilisation of problem soils, reclamation of mining areas, reforestation of over grazed areas, stabilization of sand dunes and utilization of low quality water (Aronson, 1985; Sankary & Goodin, 1985; Le Houérou, 1994).

According to Watson (1990) low quality water can be used for irrigation of *Atriplex* spp. used as a fodder crop. *Atriplex* spp. can play an important role as a protein source, produced with low quality water and from soils with low N-contents. Lange, Coleman & Cowley (1992) stress the importance of not only looking at biomass production during reforestation of arid areas but also at protein production.

One of *A. canescens*'s most common uses is for reclamation of mining areas (Senock *et al* 1991). According to Le Houérou (1994), *A. nummularia*, *A. halimus*, *A. glauca* and *A. semibaccata* can be used for reclamation and utilization of coppermine waste.

Gibeault, Meyer, Autio & Strohman (1989) found that *A. glauca* and *A. semibaccata* have the potential to serve as replacement for turf grass with a lower water requirement.

4.2 *Atriplex* as fodder source

One of the outstanding characteristics of *Atriplex* spp. is its potential as fodder resource, mainly for sheep and goats, but also for cattle and game (Wills *et al*, 1990; Mikhiel *et al*, 1992). According to Ormazabal (1991) the main reason for *Atriplex* spp. being such a valuable fodder crop is its evergreen nature which enables it to provide green feed during the dry season, and its high protein content on low potential soils. *Atriplex* spp. as fodder resource forms a major part of this study.

4.2.1 Methods of utilization

Atriplex, as fodder resource, can be utilized in two ways: as hay (mechanically cut and baled) (Watson, 1990), or as direct grazing (Leigh & Mulham, 1971).

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According to Watson, O'Leary & Glenn (1987) the following criteria are important in the selection of an *Atriplex* species or ecotype as a fodder crop: high inherent productivity and nutritive value, palatability, wide regional adaptation, rapid regrowth after grazing, extended vegetative growth period, high leaf production, and growth form suitable for grazing and/or mechanical harvest. As the plants mature, the leaf to stem ratio declines. Repeated cutting might, however, increase the proportion of useable material (leaves and young twigs) (Watson, 1990). Leigh & Mulham (1971) proposed that plants should be exposed to rotational light and heavy grazing, which will result in a compact shrub with a larger amount of young growth. Such plants would also have a better chance of surviving heavy grazing.

During hay production the following strategies are important to ensure optimum regrowth: cutting height, cutting method, baling method, cutting time and cutting frequency. During mechanical harvest leave loss is a major problem which decreases the hay quality. *Atriplex* hay can serve as roughage for mineral and protein supplementation to fodder rations (Watson, 1990; Watson & O'Leary, 1993).

On the South Island of New Zealand *A. canescens*, *A. halimus* and *A. nummularia* have been planted to serve as a fodder bank during the dry months (Wills *et al*, 1990). Other fodder crops like clover and cereals were sown between the rows of saltbush to increase the production of the fodder bank.

According to Chambers & Norton (1993), heavy spring grazing in a winter rainfall area has caused increased mortality of *A. confertifolia*. The plants also die if grazed too heavily during droughts. Light to moderate grazing should be allowed and animals should be withdrawn before active physiological growth of the plants commences.

4.2.2 Nutritive value

Watson *et al* (1987) have indicated that higher dry matter (DM) production can be associated with lower nutritive value, as the DM contains more twigs than leaves. The nutritive value of leaves is higher than that of twigs. *Atriplex* fodder production and quality varies between species, harvesting treatments and physiological stage during harvest or utilization. Protein levels of *Atriplex* spp. are high, but high ash- and NDF (neutral detergent fibre) levels, together with mineral imbalances, limit its use as the sole fodder source.

According to Colomer & Passera (1990) the crude protein (CP) content of fodder should be at least 7% to assure sufficient rumen activity. During the winter months, CP content of grasses

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is approximately 5%, in comparison with the 15 to 20% CP content of *Atriplex* species. *Atriplex* spp. can thus be planted as CP supplement for livestock, grazing poor quality standing hay. *Atriplex* spp. maintain a relatively high protein level through the year. The high N-content and the moderate OM digestibility are maintained during the drier season in contrast with the low nutritive value of perennial and annual grasses (Wilson, 1977). Leaves of *Atriplex* spp. contain higher CP and phosphorus (P) levels than grasses at comparable stages in the season. It is known that *A. canescens* is used as N-supplement for cattle, grazing low quality grass (Arthum, Holechek, Wallace, Galyean & Cardenas, 1992a).

Colomer & Passera (1990) mention that the lignin values of *Atriplex* spp. are higher than those of grasses and also that DM-digestibility varies from 60 to 70%. This digestibility is higher than those of *Eragrostis curvula* and *Acacia salicina* and the same as *Medicago arborea* and *Prosopis chilensis*. For *Atriplex* spp. the OM-digestibility , P-content, cell wall components and N levels have higher values in winter than in other seasons.

Shrubs, such as *Atriplex*, are often inferior to grasses with respect to energy values because of the higher cellulose to lignin ratio in their cell walls (Arthum, Holechek, Galyean, Cardenas & Rafique, 1992b). Wilson (1977) also mentions that the lignin composition of shrub leaves varies from that of grasses and legumes. Thus the lignin content of the acid detergent fibre does not relate to the availability of the fibre fraction for digestibility, which is the case for grasses and legumes.

The addition of *A. canescens* to a low quality hay has been compared with the addition of *Medicago sativa* L. (lucerne) by Arthum *et al* (1992b). *Atriplex canescens* caused slight changes in ruminal fermentation and digestibility in comparison with lucerne, but the changes were not significant. Watson & O'Leary (1993) replaced lucerne hay, fed to cattle and sheep, with 85% *A. barclayana* and 15% *A. deserticola*. Fodder intake did not change. The acceptability and the digestibility coefficient were significantly high. It was accepted, therefore, that baled *Atriplex* possesses the potential to serve as fodder for ruminants.

4.2.3 Nitrogen digestibility

In general, the apparent digestibility of *Atriplex* spp. is calculated on a DM basis. This might be deceptive, because the ash might include a high number of soluble salts with no energy value for livestock (Benjamin, Oren, Katz & Becker, 1992). Benjamin, Lavie, Forti, Barkai, Yonathan & Hefetz (1995) also mentioned that the apparently high *in vitro* DM digestibility of *Atriplex* spp.

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may be attributed to high (>20%) salt concentrations in the leaves. According to them, another problem is that DM digestibility is normally measured for the leaves only, while the sheep diet consists of an unknown ratio of leaves and twigs. Kessler (1990) also stresses the possibility of the DM digestibility of *Atriplex* fodder, being influenced by its high salt content.

According to Benjamin *et al* (1992) the high CP values of up to 20 % might be misleading, as up to 60% of the CP can be non protein nitrogen (NPN). This NPN is not necessary utilized by ruminants, unless high energy material is present in the rumen during the fermentation and digesting processes. Sheep, feeding on *A. nummularia* leaves, showed a negative N-balance, which changed to a positive balance after the diet was supplemented with barley grain.

According to Benjamin *et al* (1992) the mean N concentration in *Atriplex* DM is 1.66%, with an apparent digestibility of 73%. Work done by Warren, Bunny & Bryant (1990) showed that the N-content of *A. amnicola*, *A. cinerea*, *A. lentiformes* and *A. undulata* varied between 1.4 and 2.0%. The N digestibility was in the order of 70 to 81%. Crude protein levels varied from 9 to 12.4%. Wilson (1977) reported N digestibility of *A. nummularia* as 97% and of *A. vesicaria* as 93%, while it is only 50% for certain *Acacia* species. These results differ widely from those reported by Benjamin *et al* (1992) and Warren *et al* (1990). A different (older) technique might have been used by Wilson (1977).

The use of *A. amnicola* as a supplement to wheat straw resulted in high N-retention in sheep (Atiq-ur-rehman, Mackintosh, Fortune & Warren, 1994). When the diet consisted of more than 50% *A. amnicola*, 50% of the extra absorbed N was secreted through the urine. This is an indication of poor utilization of N by the micro-organisms in the rumen. This might be caused by the low metabolizable energy content of *A. amnicola* and the high lignin and ash contents of wheat straw. The apparent DM digestibility of the diet increased with an increase in *A. amnicola* in the diet. Nitrogen retention increased when only 25% *A. amnicola* was added to the wheat straw. According to Benjamin *et al* (1992), N-retention of sheep feeding on *A. barclayana* varied between 3 and 5.4g., which is equivalent to 19 - 34g CP, which is marginal for animal maintenance.

The retention of a palatable *Atriplex* spp. such as *A. canescens* in natural range, will reduce the need for protein supplementation of cattle grazing grasses during their dormant season (Arthum *et al* 1992b). Cattle will adapt their diet according to the available species. Nitrogen retention of cattle feeding on grass hay was more or less the same when supplemented with *A. canescens* as when supplemented with lucerne.

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4.2.4 Animal performance.

According to Warren *et al* (1990), sheep grazing *Atriplex* spp. need an adaptation period. Sheep lost weight during the first week, but after the second week their weight stabilised. In contrast (Jacobs & Smit, 1977) found that Merino sheep, grazing *A. nummularia*, *A. breweri*, *A. canescens* and *A. lentiformis*, maintained their weight. Wilson & Graetz (1980) compared cattle and sheep, grazing a community of *A. vesicaria* and grass. There were differences between relative mass gains of cattle and sheep. The sheep drank much less water and gave high wool production, which were the only reasons why sheep should graze this plant community rather than cattle.

During an experiment on *A. barclayana*, sheep maintained their weight. The metabolizable energy was relatively low at 6.28 $MJ.kg^{-1}DM$ (Benjamin *et al* 1992). According to Atiq-Ur-Rehman *et al* (1994), the metabolizable energy of *A. amnicola* ranges from 5 to 7 $MJ.kg^{-1}DM$. The digestible energy of *A. nummularia* has been reported as being sufficient for the maintenance requirements of goats (Colomer & Passera, 1990). A goat of 30 kg can also, meet its maintenance requirements for P by eating 525 g. DM of *A. cinerea* or *A. bunburyana* fodder. Under the same conditions, *A. cinerea* and *A. bunburyana* contain more P than *A. nummularia* and *A. undulata*. According to Watson (1990) *Atriplex* spp. have the ability to absorb selenium from the soil. Such fodder can be given as supplement to livestock grazing an area with a selenium deficiency.

5. Conclusion

The genus *Atriplex* belongs to the Chenopodiaceae family and consists of over 417 species, which are almost all halophytes and adapted to arid and semiarid conditions associated with saline soils.

The plants have an upright to prostrate growth form, are annual or perennial and are normally dioecious with single plants from some species having the ability to change sex under certain conditions. *Atriplex*, as a halophyte, has the ability to excrete excess salts through trichomes (bladder hairs) on their leaves. In a few of the species, of which *A. amnicola* is an example, microbial N-fixation was found, which might explain, together with some physiological

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phenomena, the relatively high N-levels of 1.5 - 3.0% in plants growing on N-poor soils.

Besides the wide range between species, genetic variation within a species is also found. This variation within species creates the potential for selecting ecotypes on the basis of palatability, nutritive value and productivity. One of the species with the largest genetic variation is *A. canescens*.

Lack of knowledge on seed quality and germination criteria, together with the genetic variability, hamper *Atriplex* establishment. Seed can be directly sown, or seedlings propagated from seed or cuttings can be planted. *Atriplex* spp. can be irrigated with saline water but this might influence palatability. The economical viability of *Atriplex* as planted fodder crop is uncertain, because of high input cost with low animal production. If utilized in combination with other crops and/or utilized over a long period it may be profitable.

Atriplex spp. may be used as fodder and shelter for livestock, for erosion control, the reclamation of degraded land and mining areas and fuel wood production. As a fodder crop it can be directly grazed or used for hay production. The nutritive value is high with CP values of up to 18%. Disadvantages include high ash content and low energy. The N-retention by sheep can be increased by supplementing wheat hay with *A. canescens*. Sheep will maintain their condition, feeding on *Atriplex*, although little to no animal production might be expected.

From the literature the following spp. were the most important for fodder production: *A. nummularia*, *A. canescens*, *A. halimus* and *A. vesicaria*. Other important species are: *A. amnicola*, which can tolerate high saline levels and still serve as acceptable fodder source, *A. lentiformis*, which has an extremely high biomass production, but poor leaf to stem ratio and *A. undulata*, which has a good potential for hay production because of its prostrate growth form and the ability of lateral branches to form roots when touching the soil.

The Government of South Africa recently decided to stop drought subsidies to commercial farmers. In the past the farmers depended on the Government to carry them through droughts by means of drought subsidies. In future farmers should adapt their management by looking at alternative methods to survive droughts. One such method would be to use drought tolerant

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fodder shrubs such as *Atriplex* species. In the past *A. nummularia* was studied but those findings should be refined with the possible selection and propagation of an elite cultivar, or other *Atriplex* spp. should be studied for possible utilization under South African climatological and ecological conditions.

Study objectives

From this literature study it is clear that the genus *Atriplex* has a good potential to be utilized for the reclamation of degraded land and livestock production in semi-arid and arid areas of the world. There is, however, considerable potential to select and multiply species and ecotypes with specific characteristics, needed for specific conditions. To do this, new methods of multiplication must be examined or old propagation methods must be refined. Other important aspects, requiring attention, are the selection of desirable plants using animals and also to find a way to assess the production of *Atriplex* spp. in an "on-farm" situation.

The above mentioned factors that need to be studied were investigated and are presented in this thesis. It was chosen to present each investigated aspect in article form in a separate chapter.

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CHAPTER 2

DRY MATTER PRODUCTION, STRUCTURE AND NUTRITIONAL VALUE OF TWELVE *ATRIPLEX* SPECIES

ABSTRACT

The genus *Atriplex* is widely distributed over the world and is used as fodder, for land reclamation and soil erosion control, fuel, shade and shelter. Various species were introduced to South Africa since 1889 with mixed success. Twelve species were compared in terms of dry matter (DM) production, structure, growth, *in vitro* DM digestibility (IVDMD), crude protein (CP) content, neutral detergent fibre (NDF), sodium (Na) content, percentage ash and percentage organic matter (OM). The 12 species which were compared were: *A. amnicola*, *A. breweri*, *A. canescens*, *A. cinerea*, *A. glauca*, *A. halimus*, *A. lentiformis*, *A. nummularia*, *A. paludosa*, *A. rhagadioides*, *A. semibaccata* and *A. undulata*. Plant heights varied from 0.3 m (*A. semibaccata*) to 1.35 m (*A. nummularia*). Mean percentage edible DM production was 92%, 74% and 68% at eight, 11 and 15 months after planting respectively. *A. semibaccata* yielded the highest percentage edible material at 99% with *A. nummularia* the lowest at 48%. *A. nummularia* had the highest total DM yield at 2462 g.plant^{-1} . *A. nummularia* also had the highest mean IVDMD (60%), CP (15%), Na (2.72%) and the lowest mean NDF (48%). *A. glauca* had the lowest mean IVDMD (42%) and CP (8%), with *A. canescens* (S.R.) the lowest Na (0.05%), while *A. canescens* (F.R.1) had the highest NDF (62%). It is recommended that the best accessions as fodder crops are *A. nummularia*, *A. canescens* (S.R.), *A. rhagadioides* and *A. amnicola*. The accessions with the lowest value as fodder crops were *A. semibaccata*, *A. glauca* and *A. canescens* (Rincon). These low fodder value species may however be incorporated in reclamation of degraded land and soil erosion control trials in future.

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INTRODUCTION

After three centuries of systematic evaluation, 417 species of *Atriplex* have been distinguished on the basis of floral and morphological characteristics. The plants grouped in the genus *Atriplex* are herbs and shrubs ranging in size from herbaceous annuals, of a few centimetres in height, to woody perennials, of up to five metres (Osmond, Björkman & Anderson, 1980). This genus is widely distributed all over the world and used as livestock fodder, for land reclamation and soil erosion control, as energy fuel and for landscaping, shade and shelter (Koller, Tadmor & Hillel, 1958; Aronson, 1985; Mc Kell, 1991; Squires & Ayoub, 1994). Of all the different uses of *Atriplex* the most common (important) are for reclamation of degraded land and as a fodder shrub (Wills, Begg & Brosnan, 1990).

Of the 15 *Atriplex* species indigenous to South Africa, little is known about their value as fodder shrubs. It is also difficult to determine which species are truly indigenous and which are naturalized, as quite a few were introduced in the nineteenth century. This list of indigenous species, compiled from the literature, includes: *A. albicans* (Ait), *A. amboensis* (Sching.), *A. austroafricana* (Aellen.), *A. cinerea* (Poir), *A. farinosa* (Forsk.), *A. flexuosa* (Moq.), *A. glauca* (L.), *A. littoralis* (L.), *A. patula* (Syme.), *A. perrieri* (Leandri.), *A. portulacoides* (L.), *A. rosea* (L.), *A. verreauxii* (Moq.), *A. vestita* (Thumb.) and *A. zosteraefolia* (Wats.) (Thiselton-Dyer, 1912; Osmond *et al*, 1980; Bond & Goldblatt, 1984; Shearing & van Heerden, 1994). All these species are more herbaceous than woody and don't grow higher than one metre. In South Africa, this genus has an important role to play, not only as halophyte for saline soil, but also as a drought tolerant fodder crop and for reclamation of degraded land (Le Houérou, 1994).

Previous research in South Africa was done on only a few species which were all introduced from other countries, mostly Australia. The first publication on one of the introduced species was in 1889 when Alston (1889) reported that the introduced *A. nummularia* "grew freely and seeded wonderfully", on the farm Van Wyks Vlei, which made it a better proposition than the indigenous *A. vestita*. At that stage Alston (1889) produced *A. nummularia* seed for the whole country. Since 1889 many articles have been published on *A. nummularia* in South Africa, which has made it a well known fodder shrub today. Other less well known species include: *A. semibaccata* (Burger, 1927; Spies, 1929), *A. canescens*, *A. brewerii* and *A. lentiformis* (Jacobs & Smit, 1978). Another introduced species that has become an invader in the Karoo, especially in degraded areas, is *A. lindleyi* subsp. *inflata*. This species was also introduced from Australia

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as a dual purpose shrub for erosion control and fodder. Its palatability was, however, very poor and as a result *A. lindleyi* subsp. *inflata* has become invasive in certain areas of the Karoo (Shearing & van Heerden, 1994).

Although *A. nummularia* is the most commonly used *Atriplex* species in South Africa, problems have still been experienced with palatability and establishment. The purpose of this study was to compare *A. nummularia* with other *Atriplex* species to determine whether other species were superior to *A. nummularia*. Characteristics which were compared included: dry matter (DM) production, structure, growth, *in vitro* DM digestibility (IVDMD), crude protein (CP) content, neutral detergent fibre (NDF), sodium (Na) content, percentage ash and percentage organic matter (OM). The 12 species which were compared were: *A. amnicola*, *A. breweri*, *A. canescens*, *A. cinerea*, *A. glauca*, *A. halimus*, *A. lentiformis*, *A. nummularia*, *A. paludosa*, *A. rhagadioides*, *A. semibaccata* and *A. undulata*.

MATERIAL AND METHODS

This trial was conducted on the Hatfield Experimental Farm in Pretoria. Pretoria is situated in the Gauteng Province at an elevation of 1360 m above sea level. It is a summer rainfall area with an average of 650mm per annum, concentrated in a short summer growing season. Temperatures vary from an average minimum of 11°C to an average maximum of 25°C. This is not an arid region, but at least differences under these semi-arid conditions should be evident. Soil samples were taken from eight positions within the experimental area to ensure a representative sample. The soil analysis was: 42% clay, pH(H_2O) of 5.9, Ca of 615 parts per million (ppm.), Mg of 186 ppm., P (Bray I) of 26 ppm., K of 102 ppm. and Na of 43 ppm. Except for the clay percentage, which was a bit high, it is a good cropping soil. Although the genus *Atriplex* belongs to the Chenopodiaceae family, and is adapted to saline soils with a high pH, this moderately acidic soil was acceptable as all plants would be exposed to the same soil conditions.

The twelve species which were evaluated, together with some cultivars and selections of *A. canescens*, are listed in Table 1.

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Table 1 Names and origin of *Atriplex* accessions evaluated

Accessions	Origin
A. amnicola Paul G. Wilson	Western Australia
A. canescens (Pursh.) (Australia)	Australia
A. canescens (Pursh.) (Rincon)	North America
A. canescens (Pursh.) (Santa Rita)	North America
A. canescens (Pursh.) (Field Reseve 1)	North America
A. canescens (Pursh.) Field Reseve 2)	North America
A. cinerea Poir.	Australia, Europe, South Africa
A. glauca L.	Mediterranean, South Africa
A. halimus L.	Asia, Mediterranean
A. lentiformis ssp. *breweri* (Wats.)Hall&Clem.	North America
A. lentiformis (Torr.) Wats. ssp. *lentiformis*	North America
A. nummularia L.	Australia
A. paludosa R. Br.	Australia
A. rhagadioides F. Muell.	Australia
A. semibaccata R. Br.	The Americas, Europe, Australia & South Africa
A. undulata D. Dietr.	South America

Some of the species overlap or have different scientific names amongst different scientists. According to Le Houérou (1993) *A. amnicola* is the new name for *A. rhagadioides*. This might be true as *A. amnicola* is not listed in the species list of Osmond *et al* (1980), while *A. rhagadioides* is listed. In this study both names will be used as two separate seed sources were used. At the end a comparison between these two species should demonstrate whether they are the same species or not. Another exception is *A. lentiformis* and *A. breweri*. *Atriplex breweri* is regarded as a subspecies of *A. lentiformis*, but again two separate seed sources were used and *A. lentiformis* ssp *breweri* will be referred to as *A. breweri*. A last name problem is *A. halimus* (Mediterranean saltbush). According to Osmond *et al* (1980) this name should be *A. cana* Mey. According to Osmond *et al* (1980) *A. halimus* is the new name for the South African specie *A. halimoides* or *capensis*. Again, according to Shearing & van Heerden (1994) the new name for *A. halimoides* or *capensis* is *A. vestita* which is also listed in the species list of Osmond *et al*

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(1980). Despite these confusing name changes many authors, of which Wills *et al* (1990) is just one example, still use the name *A. halimus* for Mediterranean saltbush instead of *A. cana*. In fact no other authors used *A. cana*, although there are many references to *A. halimus* as Mediterranean saltbush. Because of this confusion it was decided to use the name *A. halimus* in stead of *A. cana* for the Mediterranean saltbush.

Seedlings were produced in the greenhouse by sowing the seed in seedtrays containing a mixture of 50% sand and 50% peat. *Atriplex* seed is covered with a fruiting bract. The fruiting bract contains high concentrations of salt. It is necessary to eliminate these salts since they inhibit germination (Barnard, 1986). The seed (intact in the bracteoles - fruits) was, therefore, placed in water for 48 hours. The water was replaced with clean water twice a day. After the salts were leached from the fruiting bracts, the seed was sown. After reaching a height of 3 to 5 cm., seedlings were transferred to 0.5 litre plastic bags containing a mixture of 25% sand, 25% compost and 50% soil. The seedlings were planted in the experimental area after reaching a height of approximately 15 cm.

The experiment was a randomised block design with four replicates. Each replicate was randomly allocated. Each replicate consisted of the 16 accessions which were also randomly allocated . For each accession five plants were planted per replicate. The spacings were one metre within the row and three metres between the rows, which gave an density of 3333 plants per hectare (ha).

The seedlings were planted in the autumn. Plant heights were measured seven times over a period of 18 months. One plant of each accessions was harvested to a height of 10 cm. from each replicate, seven, 11 and 15 months after planting. The harvested material was separated into leaves, edible twigs (twigs with a diameter of four millimetres or less) and inedible twigs (diameter of more than four millimetres). This material was oven dried at 60°C to constant weight and weighed. For each of the accessions the leaves and edible twigs of all four replicates were bulked and one sample was milled for further analysis.

The following chemical analyses were conducted to determine nutritive quality of each accession: *in vitro* DM digestibility (IVDMD), using the Tilley and Terrey technique as adapted by van der Merwe (van der Merwe & Smith, 1991); neutral detergent fibre (NDF), using the Dosi Fibre system with the Robbertson and Van Soest method (Robertson & Van Soest, 1981); sodium (Na) using a wet calcination and sulphur technique; and crude protein (CP)/N-content, using the Kjeldahl technique (AOAC, 1984). Other parameters included dry matter (DM),

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organic matter (OM) and ash contents. Dry matter was determined by weighing two grams of the sample, dried at 100°C for 24 hours and weighed. Ash was determined by placing the weighed samples in an oven at 600°C for 4 hours. Organic matter (OM) was determined as the difference between DM and ash. All these analyses were done on only the edible components (leaves and edible twigs). For the determination of *in vitro* DM digestibility, NDF, CP, ash, Na and OM duplicate samples were analysed to make sure the technique was applied correctly. When the results differed more than five percent those samples were analysed again. Because all four replicates were bulked and only one sample was used for further analysis, these data were only statistically analysed for total mean values or otherwise where appropriate.

Statistical analyses, where applicable, were conducted with the ANOVA procedure to determine significant differences ($P < 0.05$). Note that for Table 8 (IVDMD) and Table 9 (NDF), the accessions that did not differ significantly were marked instead of those that did differ significantly, as there were too many significant differences to be able to indicate them all. For this the SAS (SAS, 1994) programme was used.

RESULTS AND DISCUSSION

Germination

Germination of seed from the different species varied a lot with *A. lentiformis* the highest at 80% and *A. canescens* (Santa Rita) the lowest with only 10% germination. Seed was obtained from different sources and the age of the seed was not always known. According to Silcock, Williams and Smith (1990), germination of *Atriplex* seed might decrease over time and in general seed of *Atriplex* spp. is not long lived. This might be the reason for the low germination rate of *A. canescens* (Santa Rita) as the seed was more than six years old. This characteristic of *Atriplex* seed is illustrated in Figure 1, where *A. canescens* (F.R. 2) had a higher germination rate than *A. canescens* (F.R. 1) of which the seed was a few years older. This variation in germination between the species, as seen in Figure 1, might also be caused by genetic variation within species. A species such as *A. amnicola* might have had a germination rate of more or less than the 50% obtained. According to Vlahos, Nicholas & Malcolm (1991) different seed sources (from different plant communities) of *A. amnicola*, had germination rates ranging from 0 to 75%.

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It is clear that in this experiment, only the germination rate of the tested seed sources of each species were tested and not the general germination rate for each species. To test the germination rate of a species, different seed sources with the same age group should have been used, to take the effect of age and genetic variation within species into account.

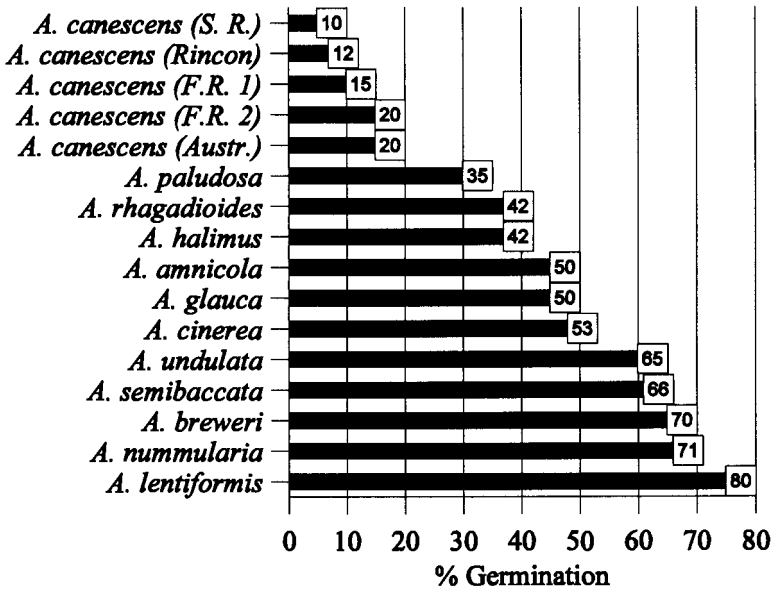

Figure 1 Percentage germination of 16 *Atriplex* accessions.

Growth

With respect to growth form the plants were divided into three groups. The first was an upright growth form typified by the five *A. canescens* ecotypes. The second group included the other five upright growing species, which were: *A. breweri*, *A. halimus*, *A. lentiformis*, *A. nummularia* and *A. paludosa*. The third group included the six species with a more prostrate growth form, namely: *A. amnicola*, *A. cinerea*, *A. glauca*, *A. rhagadioides*, *A. semibaccata* and *A. undulata*. Plant heights were measured on eight occasions until 500 days after planting. These results are presented in Figures 2, 3 and 4. The genetic difference within *A. canescens* can be seen in Figure 2 with S.R. (Santa Rita) reaching a height of almost 1.4 metres and Rincon reaching a maximum height of only 0.75 metres. According to Senock, Barrow, Gibbens & Herbel (1991) the canopy structure of *A. canescens* can vary from 1 m to more than 2 m in height.

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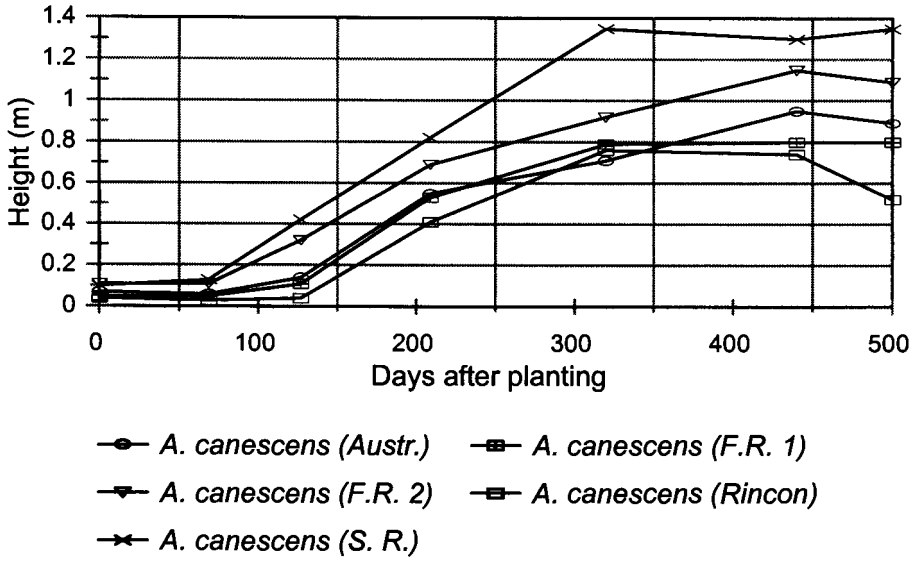

Figure 2 Plant height of the five upright growing *A. canescens* ecotypes from the day of planting until 500 days after planting.

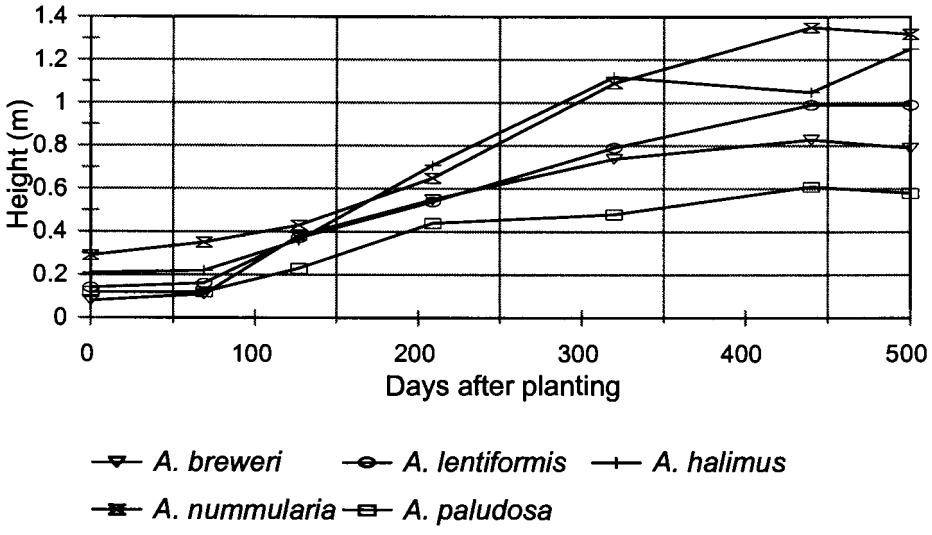

Figure 3 Plant heights of five upright growing species from the day of planting until 500 days after planting.

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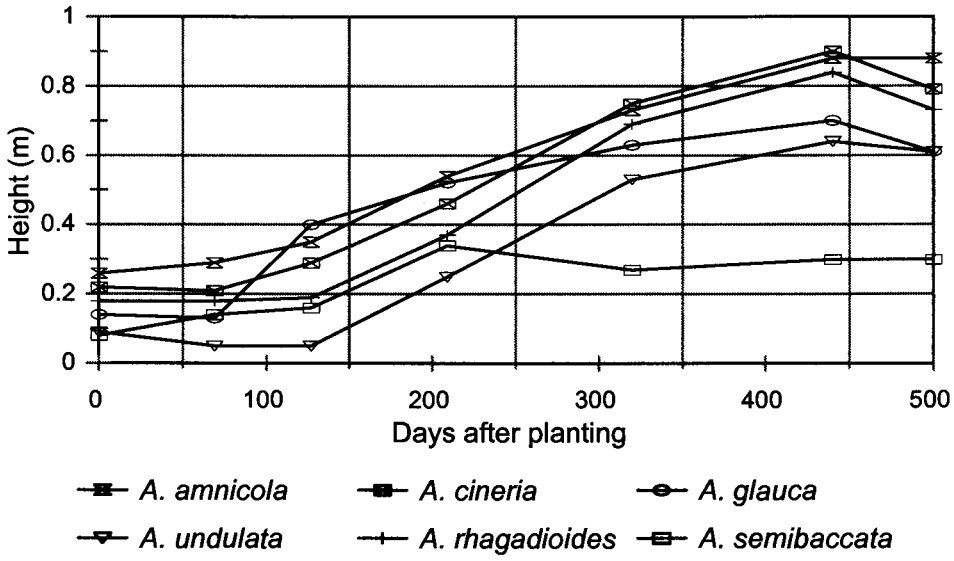

Figure 4 Plant heights of the six species with a relatively prostrate growth form, from the day of planting until 500 days after planting.

Of the other upright growing species (Figure 3), *A. nummularia* reached a height of almost 1.4 m with *A. paludosa* only reaching a height of 0.6 m. Of the species with a prostrate growth form (Figure 4), *A. amnicola* reached a height of 0.9 m and *A. semibaccata* 0.3 m. The common name for *A. semibaccata* is creeping saltbush, as it grows flat on the soil with a canopy width of over 2 metre (Table 3). This height measurement is purely relative and does not give an indication of the maximum height that each species can reach, as the time period was too short to attain maximum height. In a study by Wills *et al* (1990), *A. halimus* reached a height of 2 m, eight years after planting, which is an indication that *Atriplex* spp. can keep growing for over eight years after planting. From Figures 2 and 3 it is, however, evident that most of the rapid growth occurs in the first 350 days after planting, and that it slows down after one year (365 days). In Figure 4, illustrating the species with a relatively prostrate growth form, it was evident that with the exception of *A. semibaccata* with its creeping habit, these species continued active growth until almost 450 days after planting.

The relative volume (m^3) was also measured for each species at three stages (Table 2). The formula for the volume of a cylinder is: $v(\text{cylinder}) = \pi r^2 h$, where r = half the diameter (half of the plant width) and h = height (plant height) and $\pi = 3.143$. This should give a better indication

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of the relative size of each accession, than plant height alone.

Table 2 Relative volume (m^3) of the 16 accessions at three different stages after planting

Accessions	Volume (m^3)		
	8 months summer	11 months autumn	15 months winter
A. amnicola	0.40	1.90	2.80
A. breweri	0.40	1.66	2.50
A. canescens (Austr.)	0.52	1.57	2.47
A. canescens (F.R. 1)	0.29	1.08	1.84
A. canescens (F.R. 2)	0.30	1.68	1.09
A. canescens (Rincon)	0.08	0.86	1.18
A. canescens (S. R.)	0.49	2.98	3.92
A. cineria	0.33	1.84	2.71
A. glauca	0.94	1.51	2.22
A. halimus	0.55	3.28	2.55
A. lentiformis	0.20	1.15	1.51
A. nummularia	0.25	1.23	3.28
A. paludosa	0.17	0.44	0.65
A. rhagadioides	0.15	1.82	2.70
A. semibaccata	0.81	1.37	1.48
A. undulata	*nm	0.70	0.84

* not measured

Atriplex canescens (Santa Rita) had the same height as *A. nummularia*, but a volume of 3.92 m^3 in comparison with the 3.28 m^3 of *A. nummularia*, which indicates that the width of *A. canescens* (Santa Rita) was greater. This is clearly illustrated in Table 3, where at 15 months after planting,

Table 3 Plant height and width (m) at three stages after planting

Accessions	8 months (summer)		11 months (autumn)		15 months (winter)	
	Height	Width	Height	Width	Height	Width
A. amnicola	0.54	0.90	0.73	1.77	0.88	1.98
A. breweri	0.55	0.92	0.74	1.66	0.83	1.91
A. canescens (Austr.)	0.55	0.89	0.71	1.45	0.95	1.65
A. canescens (F.R. 1)	0.53	0.80	0.79	1.36	0.80	1.56
A. canescens (F.R. 2)	0.69	0.80	0.92	1.65	1.15	1.31
A. canescens (Rincon)	0.41	0.49	0.76	1.14	0.74	1.27
A. canescens (S. R.)	0.82	0.91	1.35	1.66	1.30	1.95
A. cineria	0.46	0.88	0.75	1.67	0.90	1.85
A. glauca	0.52	1.46	0.63	1.57	0.70	1.99
A. halimus	0.71	0.92	1.12	1.90	1.05	1.77
A. lentiformis	0.54	0.69	0.79	1.31	0.99	1.38
A. nummularia	0.65	0.70	1.09	1.17	1.35	1.76
A. paludosa	0.44	0.61	0.48	0.96	0.61	1.14
A. rhagadioides	0.37	0.65	0.69	1.83	0.84	1.98
A. semibaccata	0.34	1.72	0.27	2.50	0.30	2.50
A. undulata	*nm	nm	0.53	1.26	0.64	1.24

* not measured

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the width of *A. canescens* (Santa Rita) was 1.95 m and that of *A. nummularia* 1.76 metre. The smallest of the accessions was *A. paludosa* with an relative volume of only 0.65 m^3 at 15 months after planting.

Plants were harvested to a height of 10 cm at three intervals after planting. The survival of the plants after each harvest is presented in Table 4.

Table 4 Percentage survival of the 16 accessions after each harvest at a height of 10 to 15 cm and the average survival of harvested plants at the end of the measuring period.

Accessions	Survival percentage			Mean
	8 months summer	11 months autumn	15 months winter	
A. amnicola	100	50	50	67
A. breweri	25	0	0	8
A. canescens (Austr.)	100	50	100	83
A. canescens (F.R. 1)	75	25	100	67
A. canescens (F.R. 2)	100	25	50	58
A. canescens (Rincon)	75	75	50	67
A. canescens (S. R.)	75	75	100	83
A. cinerea	75	75	50	67
A. glauca	100	100	100	100
A. halimus	100	100	100	100
A. lentiformis	50	0	50	33
A. nummularia	100	100	100	100
A. paludosa	100	75	50	75
A. rhagadioides	100	0	100	67
A. semibaccata	100	25	100	75
A. undulata	*nm	75	75	75

* not measured

Atriplex breweri and *A. lentiformis* were very sensitive to the harvesting treatment. The survival of the other species was moderate to very good. Survival of most of the plants was poor after the 11 month harvest. This might be a result of this harvest being taken in April (autumn) and thus the beginning of the dry season. Another problem was the cutting height (10 to 15 cm) which was too harsh for the older plants. For the first harvest at eight months, this cutting height was acceptable, but should probably have been adjusted to 20 to 30 cm as the plants were getting older (bigger). According to Glenn & O'Leary (1984) 30 cm is an acceptable clipping height for *A. nummularia*, while Watson, O'Leary & Glenn (1987) suggested that clipping heights between 15 and 20 cm are acceptable for *A. lentiformis* and *A. nummularia*. Watson (1990), however, suggests that separate clipping heights should be selected for each species according to species size and growth habit.

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Seedling survival after transplanting to the field by Abdul-Halim, Al-Bardi, Yasin & Ali (1990), showed comparable survival for *A. undulata*, *A. semibaccata*, and *A. paludosa*, but for *A. lentiformis* they got 100% survival compared with 33% in this experiment and for *A. halimus* and *A. nummularia* they got 36% and 19% respectively in comparison with the 100% for each in this trial. The soil on which the experiment of Abdul-Halim *et al* (1990) was planted, had a high salt content. A possible reason for the differences in our results might, therefore, be that *A. lentiformis* has a much higher salt requirement than *A. halimus* and *A. nummularia*. Another reason is that the plants were not harvested as in our trial, where the plants were harvested, and not all the species had the same ability to recover.

Dry matter production and structure

Plants were harvested at eight, 11 and 15 months after planting. At each harvest, the plants were separated into leaves, edible twigs and inedible twigs. Total DM-production per plant was measured for each accession and also the contribution of each component to the total.

The results for each harvest are illustrated in Tables 5, 6 and 7. These illustrate the DM-production ($g.plant^{-1}$) of each accession as well as the percentage contribution of leaves, edible- and inedible twigs to the total DM-production. At the first harvest, eight months after planting, the plants were still young and herbaceous and were only divided into edible material and inedible material, where the inedible material consist of all twigs with a diameter larger than 4 mm, and the edible material the rest. At this stage, *A. undulata* was still too small to be harvested and is, therefore, not included in this data set. For the other two harvests, all the species were harvested and separated into leaves, edible twigs and inedible twigs, as can be seen in Tables 6 and 7. Note that in the tables, each accession is marked with a alphabet letter which is used to indicate which accessions differed significantly from each other.

From Table 5 (eight months after planting) it is evident that the plants were still young with low total DM-production and high proportion of edible component, as the young growth is herbaceous and only a small proportion of each plant is woody material. *Atriplex halimus* gave the highest proportion inedible material (18%) while *A. canescens* (Rincon) and *A. semibaccata* had 0% inedible material. *Atriplex glauca* and *A. semibaccata* had significantly ($p< 0.05$) more DM.

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Table 5 DM yield (g.plant^{-1}) of the edible and inedible components, and the percentage contribution to the total yield, eight months after planting.

Accessions	Edible	Inedible	Total	Inedible	Edible
	g.plant^{-1}			%	
(a) *A. amnicola*	225 ± 242 ^{f}	21 ± 32	246 ± 274 ^{f}	6	94 ± 4 ^{j}
(b) *A. breweri*	262 ± 126 ^{f,n}	27 ± 29	289 ± 154 ^{f,n}	7	93 ± 6
(c) *A. canescens* (Austr.)	166 ± 85	26 ± 28	192 ± 106	11	89 ± 11 ^{f,o}
(d) *A. canescens* (F.R. 1)	163 ± 53	5 ± 6 ^{i,j}	168 ± 57	2	98 ± 3 ^{g,j,l}
(e) *A. canescens* (F.R. 2)	172 ± 61	5 ± 6 ^{i,j}	177 ± 64	2	98 ± 3 ^{g,j,l}
(f) *A. canescens* (Rincon)	22 ± 7 ^{a,b,g,j,l}	0 ± 0 ^{i,j}	22 ± 7 ^{a,b,g,j,l}	0	100 ± 0 ^{c,g,j,l,m}
(g) *A. canescens* (S. R.)	256 ± 51 ^{f,n}	41 ± 28	297 ± 53 ^{f,n}	14	86 ± 9 ^{d,e,f,n,o}
(h) *A. cineria*	192 ± 187	9 ± 13 ^{i,j}	200 ± 200	6	94 ± 7 ^{j}
(i) *A. glauca*	621 ± 294 *	66 ± 87 ^{a,d,e,f,h,k,m,n,o}	687 ± 340 *	7	93 ± 10
(j) *A. halimus*	255 ± 91 ^{f,n}	56 ± 35 ^{d,e,f,h,k,n,o}	311 ± 122 ^{f,n}	17	83 ± 6 ^{a,d,e,f,h,n,o}
(k) *A. lentiformis*	110 ± 60	8 ± 3 ^{i,j}	118 ± 62	7	93 ± 2
(l) *A. nummularia*	257 ± 112 ^{f,n}	39 ± 19	296 ± 129 ^{f,n}	13	87 ± 2 ^{d,e,f,n,o}
(m) *A. paludosa*	92 ± 85	14 ± 23 ^{i}	106 ± 86	12	88 ± 18 ^{f,n,o}
(n) *A. rhagadioides*	36 ± 17 ^{b,f,g,l}	1 ± 1 ^{j}	37 ± 18 ^{b,g,j,l}	1	99 ± 2 ^{g,j,l,m}
(o) *A. semibaccata*	968 ± 164 *	0 ± 0 ^{i}	968 ± 164 *	0	100 ± 0 ^{c,g,j,l,m}
Average % component contribution of all the species				7	93

Superscript: indicates values that differed significantly ($p<0.05$) from other accessions, in the same column, marked with the same letter

*For the edible and total DM, *A. glauca* and *A. semibaccata* differed significantly from all the other accessions

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These two species (*A. glauca* and *A. semibaccata*) both have a prostrate to creeping growth form which clearly distinguishes them from many of the other species. Gibeault, Meyer, Atio & Strohman (1989) found that these two species performed better than clover (*Trifolium* spp.), fescue (*Festuca* spp.) and some other grass species, at a young growth stage, under low soil moisture regimes. Total yields of *A. canescens*, *A. cinerea* and *A. nummularia* were comparable with the results of Watson (1990) for the same species at more or less the same age. *Atriplex lentiformis*, however, yielded only 118 g.plant^{-1} in this experiment, while it produced 340 g.plant^{-1} in Watson's experiment. This higher yield could be a result of the plants being irrigated with saline water. *Atriplex lentiformis* possesses the ability to give extremely high yields (much higher than *A. nummularia*) when irrigated with saline water (Watson *et al*, 1987). In this experiment the plants received only precipitation, apart from being irrigated with fresh water during and two weekly after establishment until the first rainfall in spring.

At 11 months after planting (Table 6) the proportion of the edible component had declined and the inedible component increased. The contributions of inedible and edible components at eight and 11 months were on average 8% versus 28% and 92% versus 72% respectively. With respect to total DM-production, *A. canescens* (Santa Rita), *A. halimus*, *A. nummularia*, *A. rhagadioides* and *A. semibaccata* had yields of 1200 g.plant^{-1} and more. *Atriplex canescens* (Santa Rita) and *A. nummularia* had the highest percentage of inedible material.

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Table 6 DM yield (g.plant^{-1}) of the edible and inedible components, and the percentage contribution to the total yield, 11 months after planting.

Accessions	Leaves	Twigs		Total	Leaves	Twigs		Total
		Edible	Inedible			Edible	Inedible	Edible
	g.plant^{-1}				%			
(a) *A. amnicola*	303 ± 119	523 ± 384 ^{f,k,l,m,p}	264 ± 304 ^{l}	1090 ± 782 ^{f,m,p}	30	51	19	81 ± 12 ^{c,g,f,j,k,l,o}
(b) *A. breweri*	284 ± 62	440 ± 153 ^{m}	284 ± 68 ^{l}	1008 ± 277	28	44	28	72 ± 2 ^{h,l,n,o}
(c) *A. canescens* (Austr.)	383 ± 179 ^{f,k}	227 ± 106 ^{n}	317 ± 142	927 ± 408 ^{g}	42	25	32	68 ± 5 ^{a,h,i,m,n,o}
(d) *A. canescens* (F.R. 1)	230 ± 114 ^{j,l}	308 ± 101	236 ± 169 ^{l}	774 ± 448 ^{g}	29	38	33	67 ± 9 ^{h,i,l,m,n,o}
(e) *A. canescens* (F.R. 2)	292 ± 127	308 ± 77	329 ± 230 ^{o}	930 ± 415 ^{g}	31	34	34	66 ± 6 ^{h,i,l,m,n,o}
(f) *A. canescens* (Rincon)	97 ± 55 ^{c,j,l,n,o}	120 ± 95 ^{a,i,n}	122 ± 95 ^{l}	339 ± 239 ^{a,g,j,l,n,o}	30	37	34	66 ± 8 ^{a,h,i,l,m,n,o}
(g) *A. canescens* (S. R.)	639 ± 206 *	350 ± 89	704 ± 325 ***	1692 ± 560 ^{d,c,e,f,h,k,m,p}	39	22	40	60 ± 8 ^{a,h,i,m,n,o,p}
(h) *A. cineria*	264 ± 139	312 ± 242	60 ± 55 ^{j,l}	637 ± 427 ^{g,o}	43	50	7	93 ± 6 ^{b,c,d,e,f,g,j,k,l}
(i) *A. glauca*	288 ± 160	528 ± 439 ^{f,k,l,m,p}	306 ± 276	1123 ± 839 ^{p}	30	53	17	83 ± 12 ^{c,d,e,f,g,j,k,l,o}
(j) *A. halimus*	476 ± 161 ^{d,f,k,m,p}	378 ± 223	402 ± 206 ^{h,m,o,p}	1257 ± 184 ^{f,k,m,p}	37	29	34	66 ± 18 ^{a,h,i,m,n,o}
(k) *A. lentiformis*	139 ± 57 ^{c,j,l,n,o}	194 ± 76 ^{a,i}	200 ± 113 ^{l}	533 ± 229 ^{g,j,o}	27	37	35	65 ± 7 ^{a,h,i,m,n,o}
(l) *A. nummularia*	456 ± 241 ^{d,f,k,m,p}	180 ± 91 ^{a,i}	573 ± 274 ^{a,b,d,f,h,k,m,n,o,p}	1209 ± 599 ^{f,m,p}	37	15	48	52 ± 3 ^{a,b,d,e,f,h,i,m,n,o,p}
(m) *A. paludosa*	190 ± 115 ^{j,l,o}	96 ± 72 ^{a,b,i,n}	70 ± 49 ^{j,l}	357 ± 224 ^{a,g,j,l,n,o}	56	28	16	84 ± 11 ^{c,d,e,f,g,j,k,l,o}
(n) *A. rhagadioides*	404 ± 155 ^{f,k,p}	578 ± 310 ^{c,f,m,p}	216 ± 321 ^{l}	1199 ± 673 ^{f,m,p}	36	51	13	87 ± 15 ^{b,c,d,e,f,g,j,k,l}
(o) *A. semibaccata*	425 ± 301 ^{f,k,m,p}	943 ± 443 **	16 ± 15 e,^{j,l}	1383 ± 703 ^{f,h,k,m,p}	31	68	1	99 ± 1 ^{a,b,c,d,e,f,g,i,j,k,l,n,p}
(p) *A. undulata*	150 ± 84 ^{j,l,n,o}	128 ± 41 ^{a,i,n}	75 ± 101 ^{j,l}	353 ± 222 ^{a,g,j,i,l,n,o}	43	36	20	80 ± 14 ^{g,l,o}
Average % component contribution of all the species							26	74

Superscript: indicates values that differed significantly ($p<0.05$) from other accessions, in the same column, marked with the same letter

* *Atriplex canescens* (S.R.) differs significantly ($p<0.05$) from all the accessions except from *A. halimus* and *A. nummularia*

** *Atriplex semibaccata* differs from all the other accessions

*** *Atriplex canescens* (S.R.) differs significantly ($p<0.05$) from all the accessions except from *A. nummularia*

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At 15 months after planting (Table 7) the proportion of edible component (67%) had declined even further, while the inedible component (33%) had increased. The following species had a total dry matter yield of 2000 g.plant^{-1} or more: *A. amnicola*, *A. canescens* (Santa Rita), *A. glauca*, *A. nummularia* and *A. rhagadioides*. From this it is clear that the total DM yield increases with age, but the percentage edible material decreases. The plants are getting bigger, but more woody with a smaller percentage available for browsing by livestock. The total edible component is, however, significantly higher 15 months after planting than 11 months after planting for all the species with the exception of *A. canescens* (Australia), *A. canescens* (F.R. 2), *A. canescens* (Rincon) and *A. halimus*. This phenomenon in *A. canescens* is not unusual as it is the *Atriplex* species with the most distinct geographical, ecological and morphological differences (Senock *et al*, 1991).

In this experiment, different plants were harvested at each age and it might be true that, for the above mentioned *A. canescens* accessions, the different plants within the accessions are not strongly enough genetically related to be compared. Only four replicates were harvested per accession. The harvesting of more plants per accession might have given other results. The edible component for *A. halimus* was lower at 15 months, than at 11 months, while the total DM-yield was significantly higher, indicating that *A. halimus* is becoming more woody with time and is producing less edible material.

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Table 7 DM yield (g.plant^{-1}) of the edible and inedible components, and the percentage contribution to the total yield, at 15 months after planting

Accessions	Leaves	Twigs		Total	Leaves	Twigs		Total
		Edible	Inedible			Edible	Inedible	Edible
	g.plant^{-1}				%			
(a) *A. amnicola*	781 ± 202 ^{b,c,d,e,f,h,i,j,k,o,p}	790 ± 295 ^{e,f,j,k,m,p}	551 ± 235 ^{e,f,m,o,p}	2122± 641 ^{d,e,f,k,m,p}	38	37	25	75 ± 7 ^{b,f,g,j,k}
(b) *A. breweri*	305 ± 136 ^{a,g,n}	743 ± 169 ^{f,k,m,p}	689 ± 310 **	1738± 573 ^{e,f,k,p}	18	43	39	61 ± 6 ^{a,h,i,m}
(c) *A. canescens* (Austr.)	227 ± 91 ^{a,g,l,m,n}	415 ± 173 ^{i,n}	680 ± 201 ^{d,e,f,h,k,m,n,o,p}	1322± 391 ^{f,g,l}	17	31	52	48 ± 7 #
(d) *A. canescens* (F.R. 1)	261 ± 25 ^{a,g,l,n}	450 ± 170 ^{i,n}	353 ± 187 ^{c,o}	1064± 336 ^{a,g,i,l,n}	25	43	32	68 ± 7 ^{g,h,i,j,k}
(e) *A. canescens* (F.R. 2)	172 ± 57 ^{a,g,l,n}	354 ± 109 ^{a,h,i,n}	242 ± 129 ^{a,c}	769 ± 281 ^{a,b,g,j,i,l,n}	23	47	30	70 ± 6 ^{g,h,j,k}
(f) *A. canescens* (Rincon)	55 ± 27 ^{a,g,i,j,l,m,n}	159 ± 121 ^{a,b,h,i,n}	192 ± 226 ^{a,c}	406 ± 374 ^{a,b,c,g,i,j,l,n,o}	16	44	40	60 ± 10 ^{a,h,i,m,p}
(g) *A. canescens* (S. R.)	739 ± 221 ^{b,c,d,e,f,h,k,o,p}	467 ± 79 ^{i,n}	1183± 384 ***	2389± 647 ^{c,d,e,f,k,m,p}	31	20	49	51 ± 3 ^{a,d,e,h,i,m,p}
(h) *A. cineria*	360 ± 67 ^{a,g,l,n}	761 ± 349 ^{f,j,k,m,n,p}	251 ± 153 ^{c}	1372± 496 ^{l}	27	56	17	83 ± 6 ^{b,d,e,f,g,i,k,p}
(i) *A. glauca*	454 ± 196 ^{a,f,k,n}	1132± 340 ^{c,d,e,f,g,j,k,l,m,p}	408 ± 199 ^{o}	1995± 412 ^{d,e,f,k,m,p}	23	57	20	80 ± 8 ^{b,d,f,g,j,k}
(j) *A. halimus*	488 ± 146 ^{a,e,f,k,p}	356 ± 166 ^{a,h,i,n}	769 ± 366 ****	1613± 539 ^{e,f,k,l,p}	31	22	47	53 ± 11 ^{a,d,e,h,i,m,p}
(k) *A. lentiformis*	123 ± 52 ^{a,g,j,i,l,m,n}	279 ± 129 ^{a,b,h,i,n}	307 ± 158 ^{c}	709 ± 312 ^{a,b,g,i,j,l,n,o}	18	40	42	58 ± 9 ^{a,d,e,h,i,m,p}
(l) *A. nummularia*	752 ± 359 ^{c,d,e,f,h,k,n,o,p}	441 ± 161 ^{i,n}	1268± 146 *****	2462± 597 ^{c,d,e,f,h,j,k,m,o,p}	30	17	53	47 ± 8 ##
(m) *A. paludosa*	570 ± 491 ^{c,e,f,k,p}	264 ± 212 ^{a,b,h,i,n}	231 ± 152 ^{a,c}	1064± 829 ^{a,g,i,l,n}	53	24	23	77 ± 5 ^{b,f,g,j,k}
(n) *A. rhagadioides*	733 ± 394 ^{b,c,d,e,f,h,i,k,l,o,p}	1002± 742 ^{c,d,e,f,g,j,k,l,m,p}	269 ± 172 ^{c}	2003± 1304 ^{d,e,f,k,m,p}	37	49	14	86 ± 1 ###
(o) *A. semibaccata*	333 ± 130 ^{a,g,l,n}	1219± 357 *	14 ± 16 ^{a,c,d,i}	1566± 465 ^{f,k,l,p}	21	78	1	99 ± 1####
(p) *A. undulata*	168 ± 131 ^{a,g,j,l,m,n}	233 ± 114 ^{a,b,h,i,n}	168 ± 139 ^{a,c}	569 ± 361 ^{a,b,g,i,j,l,n,o}	29	42	29	71 ± 10 ^{f,g,h,j,k}
Average % component contribution of all the species							32	68

Superscript: indicates values that differed significantly ($p<0.05$) from other accessions, in the same column, marked with the same letter

* *Atriplex semibaccata* differs significantly ($p<0.05$) from all the accessions except from *A. glauca* and *A. rhagadioides*

** *Atriplex breweri* differs significantly ($p<0.05$) from all the accessions except from *A. amnicola*, A. *canescens* (Austr.), *A. glauca* and *A. halimus*

*** *Atriplex canescens* (S.R.) differs significantly ($p<0.05$) from all the accessions except from *A. nummularia*

**** *Atriplex halimus* differs significantly ($p<0.05$) from all the accessions except from *A. amnicola*, *A. breweri* and *A. canescens* (S.R.)

***** *Atriplex nummularia* differs significantly ($p<0.05$) from all the accessions except from *A. canescens* (S.R.)

Atriplex canescens (Austr.) differs significantly ($p<0.05$) from all the accessions except from *A. canescens* (S.R.), *A. halimus*, *A. lentiformis* and *A. nummularia*

Atriplex nummularia differs significantly ($p<0.05$) from all the accessions except from *A. canescesns* (Austr.), *A. canescens* (S.R.) and *A. halimus*

Atriplex semibaccata differs significantly ($p<0.05$) from all the accessions

Atriplex rhagadioides differs significantly ($p<0.05$) from *A. cineria*, *A. glauca* and *A. paludosa*

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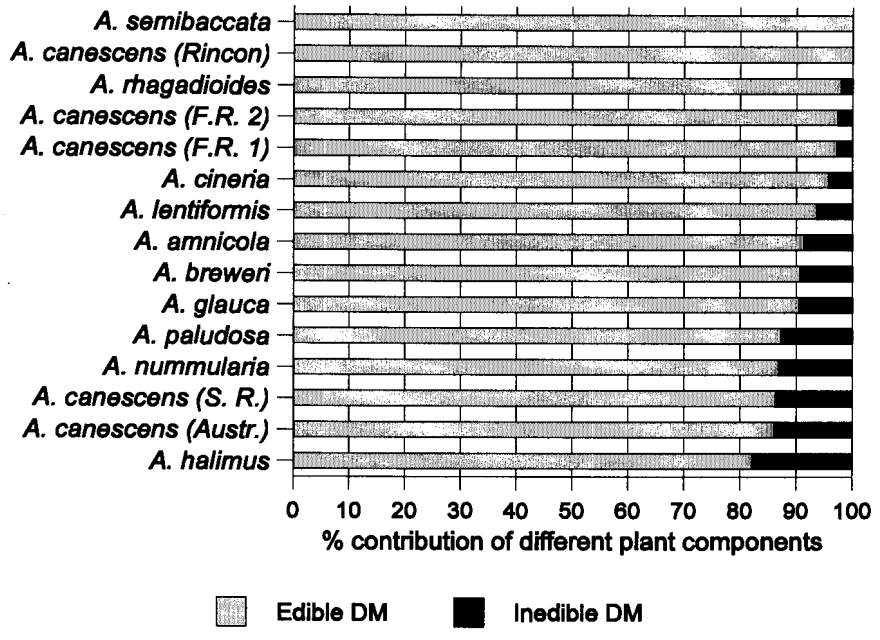

Figure 5 Percentage contribution of the different plant components of each accession, 8 months after planting.

Concerning the relative contribution of the different plant components to total plant yields this is clearly illustrated in Figures 5, 6 and 7, where data is arranged from those accessions with the lowest inedible component to those accessions with the highest inedible component.

In Figure 5 the edible component was not divided into edible twigs and leaves, as the plants were still small and herbaceous. In all three Figures, *A. canescens* (Australia), *A. canescens* (Santa Rita), *A. halimus* and *A. nummularia* stand out as the species with the highest proportion inedible material.

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When looking at leaf production in Figures 6 and 7, *A. paludosa* has the highest proportion of leaves. In Figure 7 it can be seen that this species has more than 50% leaves, 15 months after planting in comparison with *A. canescens* (Rincon) which had only 10 % leaves. Other species with a high proportion of leaves are *A. amnicola*, *A. cineria*, *A canescens* (Australia), *A. rhagadioides* and *A. undulata* (Figures 6 and 7). From Figures 6 and 7 it is clear that the proportion of leaves 15 months after planting was much lower than 11 months after planting for all species except, *A. amnicola* and *A. paludosa* which stayed virtually the same. This might be a result of the harvest at 15 months after planting being in mid winter, which is also the dry season. Together with frost damage visible leaf loss was evident.

Watson *et al* (1987) also found that the proportion of leaves decreased while the proportion of stems (twigs) increased over time for *A. lentiformis* and *A. nummularia*, with *A nummularia* producing an higher proportion of leaf DM than *A. lentiformis*. For plants, a few months older than the plants in this trial (15 months), Benjamin, Lavie, Forti, Barkai, Yonathan & Hefetz (1995) found the percentage leaf of *A. canescens* and *A. nummularia* to be 28% and 32% respectively. These results are in line with the 21% (average of all five *A. canescens* accessions) and 31% for *A. nummularia* in this experiment.

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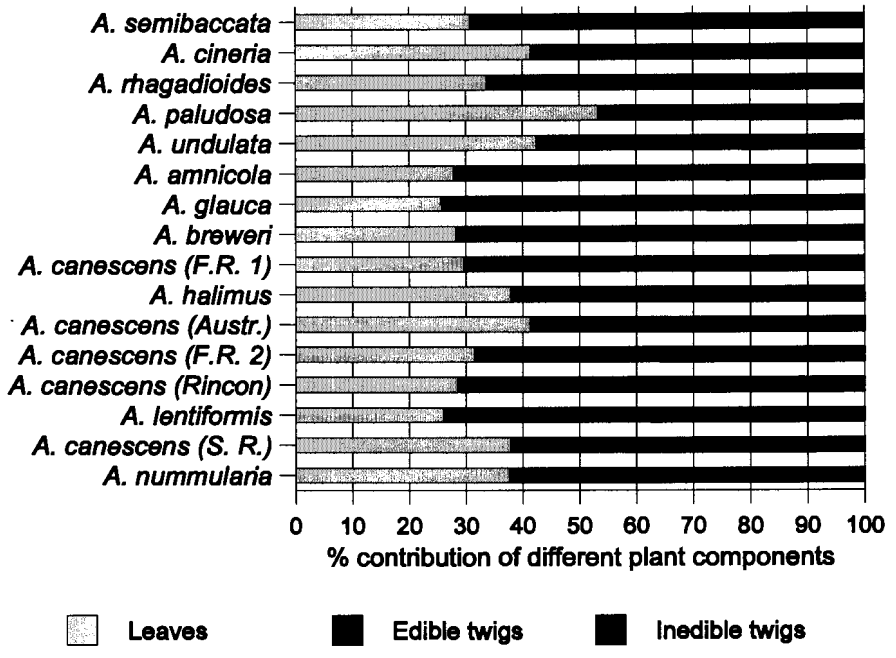

Figure 6 Percentage contribution of the different plant components to the total DM-production of each accession, 11 months after planting

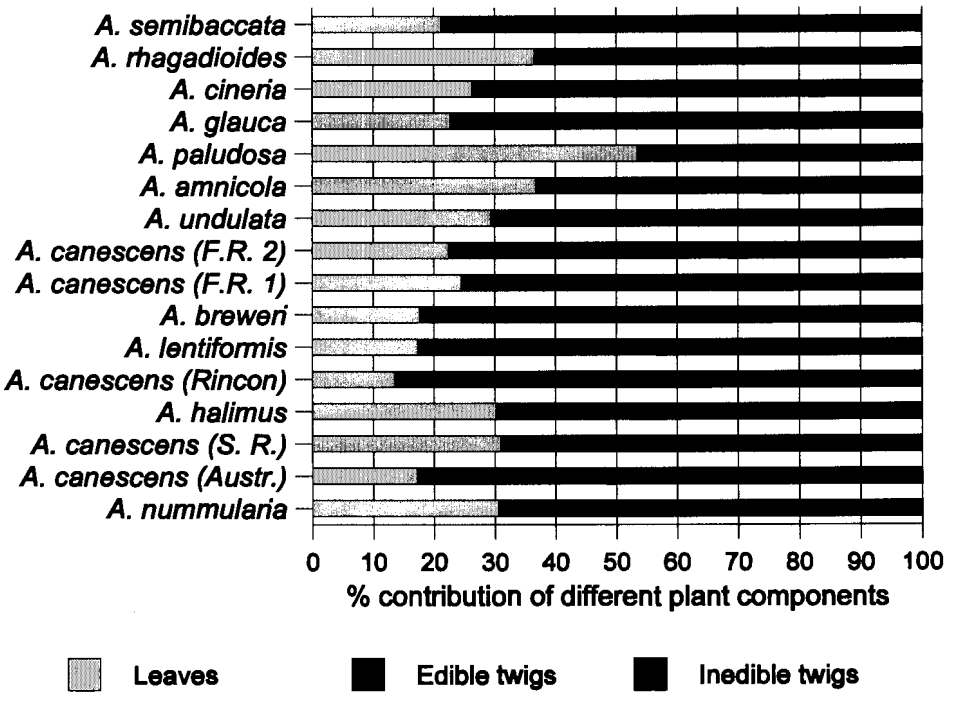

Figure 7 Percentage contribution of the different plant components to the total DM-production of each accession, 15 months after planting

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In Figures 8, 9 and 10 the total edible (leaves plus edible twigs) DM-production is illustrated, for the three harvests as this is the important component for animal production. Total DM-production does not give any indication of the DM available for animal production. From Figure 8 (eight months after planting) it is evident that *A. glauca* and *A. semibaccata* had the highest yields of 2.07 and 3.23 t.ha^{-1} respectively. The other species differed little with *A. canescens* (Rincon) and *A. rhagadioides* having the lowest yields.

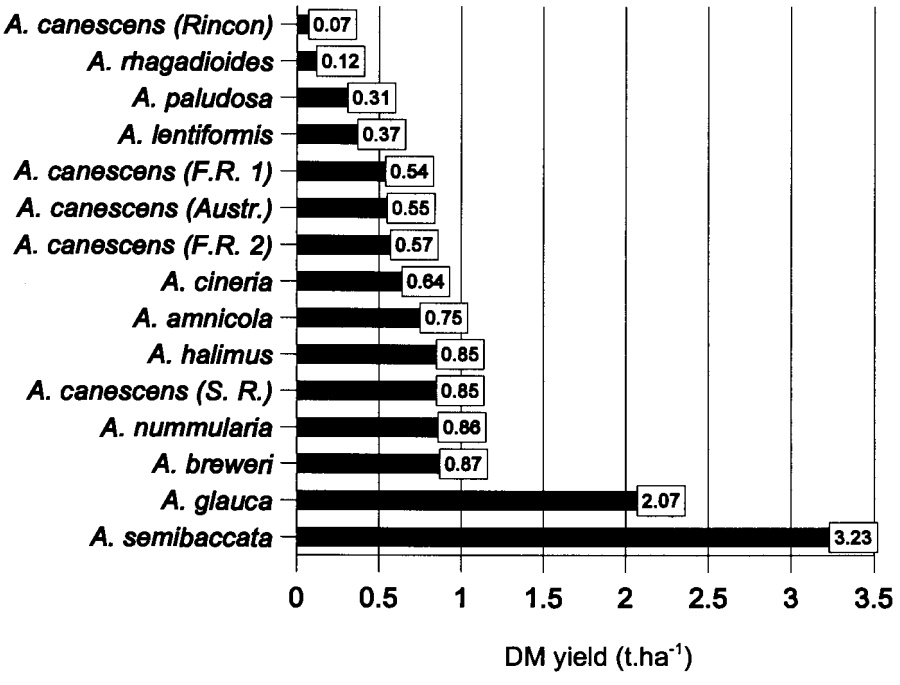

Figure 8 Dry matter yield (ton per hectare) of the edible components (leaves plus edible twigs) of 15 accessions, eight months after planting, at a density of 3333 plants per hectare.

From Figure 9 (11 months after planting), the following species yielded more than 2.7 t.ha^{-1}: *A. glauca*, *A. amnicola*, *A. halimus*, *A. rhagadioides*, *A. canescens* (Santa Rita) and *A. semibaccata* the highest with 4.56 t/ha. At this time *A. rhagadioides* gave the third highest yield in comparison with the second lowest yield at the younger stage (8 months). *Atriplex canescens* (Rincon), *A. undulata* and *A. paludosa* still had yields of less than 1 t.ha^{-1}.

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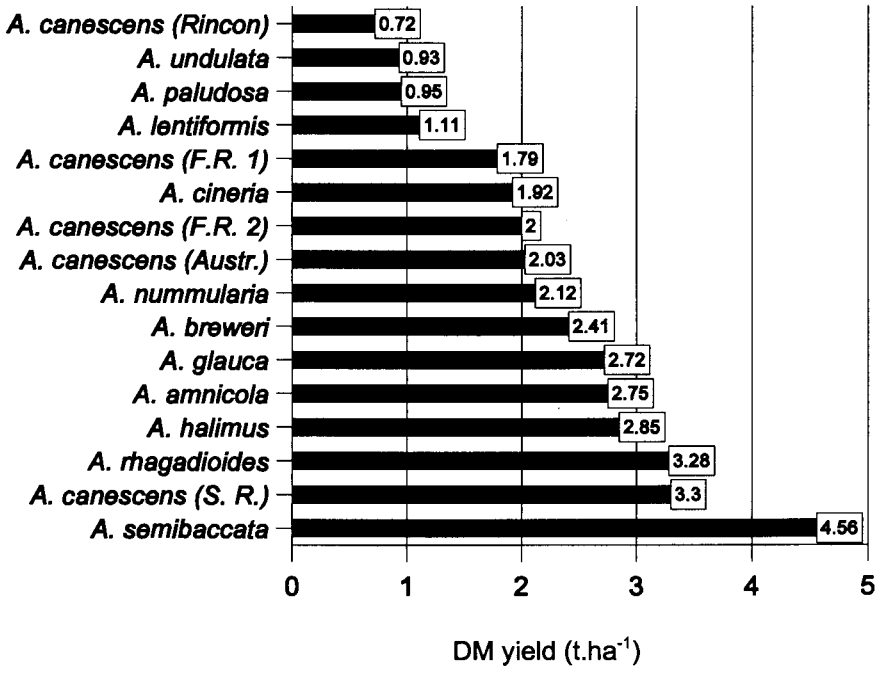

Figure 9 Dry matter yield (ton per hectare) of the edible components (leaves plus edible twigs) of 16 accessions, 11 months after planting, at a density of 3333 plants per hectare.

At 15 months after planting (Figure 10), the following plants gave yields higher than 5 t.ha^{-1}: *A. semibaccata*, *A. amnicola*, *A. glauca* and *A. rhagadioides*. The plants with yields less than 1.5 t.ha^{-1} were *A. lentiformis* and *A. undulata* with *A. canescens* (Rincon) still yielding less than 1 t.ha^{-1}. *Atriplex canescens* (Santa Rita) and *A. nummularia* both yielded close to 4 t.ha^{-1} edible DM as can be seen in Figure 10. This relatively poor yield of edible DM of these two species in comparison with the high yields of *A. semibaccata*, *A. amnicola*, *A. glauca* and *A. rhagadioides*, stresses the importance of looking at edible DM production rather than total DM production when plants are evaluated as fodder source for livestock.

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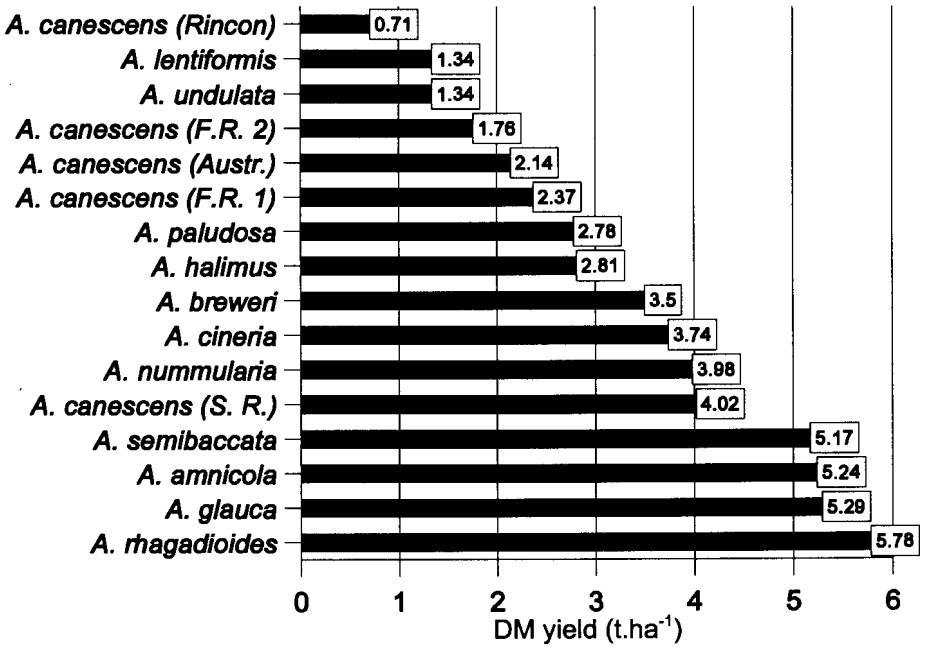

Figure 10 Dry matter yield (ton per hectare) of the edible components (leaves plus edible twigs) of 16 accessions, 15 months after planting, at a density of 3333 plants per hectare.

When looking at total DM production, *A. canescens* (Santa Rita) and *A. nummularia* gave significantly ($p<0.05$) higher yields than almost all the other accessions (Table 7). Another important factor is the nutritive value of the edible DM component. In Figure 11 the CP-production (kg/ha) of the 16 accessions at 15 months after planting is illustrated. The same material that were harvested to determine DM-production and structure was analysed for N-content, which were multiplied by 6.25 to obtain the CP-value. From Figure 11, it is evident that *A. rhagadioides*, *A. amnicola*, *A. nummularia* and *A. canescens* (Santa Rita) had the highest CP-yields. This is very important because although *A. glauca* and *A. semibaccata* had a noticeable higher edible DM production than *A. canescens* (Santa Rita) and *A. nummularia*, their CP-yields were much lower. Factors such as *in vitro* digestibility and fibre content will also influence the nutritive value and will be discussed later in this chapter.

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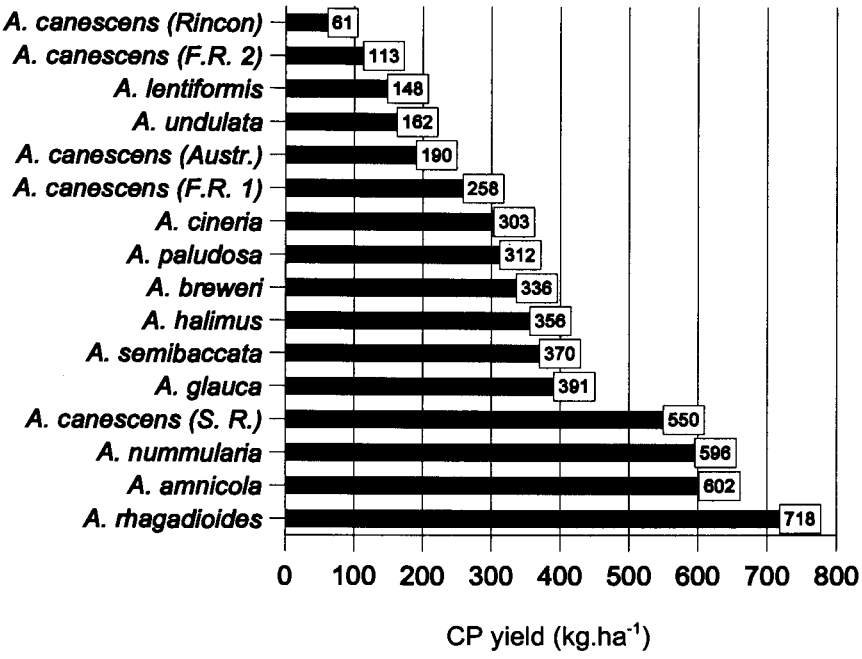

Figure 11 Relative crude protein (CP) yields (kg.ha^{-1}) of the edible components, of the 16 accessions, 15 months after planting.

In vitro DM digestibility, fibre and crude protein

In order to compare the nutritive values of the different accessions, *in vitro* digestibility, crude protein (CP) and neutral detergent fibre (NDF) were determined. The leaves and edible twigs were measured separately to see if the leaf to stem ratio would have a big influence on the nutritive value for livestock of these accessions.

In vitro DM digestibility

The results of the *in vitro* DM digestibility of the different edible components can be seen in Table 8. Digestibility of the leaves was much higher than that of edible twigs. The digestibility of the twigs was generally higher 15 months after planting, while the leaf digestibility did not vary much. This might be due to change in season from autumn to winter. The same can be seen for the *in vitro* DM digestibility of the total edible component which changed from 56% (8

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months after planting, season:summer), 60% (11 months after planting, season: autumn) to 63% (15 months after planting, season: winter) for *A. nummularia* (Table 8). For *A. amnicola* it changed from 48% to 42% to 59%.

Table 8 Percentage *in vitro* (DM) digestibility of the edible components at three stages. Total indicating the percentage *in vitro* digestibility of total edible component at each stage and was compiled from the proportion of leaves and twigs in the total edible DM yield.

Accessions	8 months	11 months			15 months			Total
	Total	Leaves	Twigs	Total	Leaves	Twigs	Total	Mean
(a) *A. amnicola*	48 ^{f,h,j,k,n,o}	73	24	42 ^{d,e,f}	81	37	59 ^{j,n}	53
(b) *A. breweri*	61 ^{c,e}	74	22	42 ^{d,e,f}	71	34	45 ^{h,i,k,o}	49
(c) *A. canescens* (Austr.)	57 ^{b,d,l,m}	66	19	49 ^{h,k,n,p}	75	24	42 ^{e,h,k}	49
(d) *A. canescens* (F.R. 1)	54 ^{c,f,l,m,o}	71	11	40 ^{a,b,i,o}	78	37	52	49
(e) *A. canescens* (F.R. 2)	63 ^{b,g}	74	16	44 ^{a,b,f,n}	70	26	41 ^{c,h}	49
(f) *A. canescens* (Rincon)	51 ^{a,d,h,j,k,n,o}	73	20	44 ^{a,e,b}	73	24	37	44
(g) *A. canescens* (S. R.)	65 ^{e}	75	17	54 ^{j,m}	77	24	56 ^{n,p}	58
(h) *A. cinerìa*	48 ^{a,f,j,k,n,o}	73	26	48 ^{c,k,n,p}	69	30	43 ^{b,c,e,i,k}	46
(i) *A. glauca*	43	72	23	40 ^{d,o}	75	32	44 ^{b,h,k,o}	42
(j) *A. halimus*	47 ^{a,f,h,k,n}	72	27	52 ^{g}	78	31	58 ^{a,n}	52
(k) *A. lentiformis*	48 ^{a,f,h,j,n,o}	74	28	47 ^{c,h,n,p}	76	29	43 ^{b,c,h,i,o}	46
(l) *A. nummularia*	56 ^{c,d,m}	71	34	60	69	52	63	60
(m) *A. paludosa*	56 ^{c,d,i}	71	28	57 ^{g}	80	38	67	60
(n) *A. rhagadioides*	50 ^{a,f,h,j,k,o}	75	27	47 ^{c,e,h,k,n}	83	38	57 ^{a,g,j,p}	51
(o) *A. semibaccata*	52 ^{a,d,f,h,k,n}	69	25	39 ^{d,i}	70	38	45 ^{b,i,k}	45
(p) *A. undulata*	*nm	69	25	49 ^{c,h,k,p}	74	42	55 ^{g,n}	52

* not measured

Superscript: indicates values that **did not** differ significantly ($p<0.05$) from other accessions, in the same column, marked with the same letter

It is difficult to find a specific pattern of change for all the species, except that for most of the species the *in vitro* digestibilities of the total edible components were the lowest in autumn, 11 months after planting.

The *in vitro* digestibility of leaves ranged from 66% to 82%. The digestibility of *A. nummularia* leaves (71% and 69%) was similar to that reported (69% and 73%) by Wilson (1977) and Benjamin *et al* (1995) respectively. For *A. canescens* leaves Benjamin *et al* (1995) reported a digestibility of 62% which is lower than the values (>70%) reported in this experiment. Benjamin, Oren, Katz and Becker (1992) found the *in vitro* digestibility of *A. barclayana* leaves to be 59% which is lower than any values in this experiment. *In vitro* digestibility values for leaves and twigs of *A. amnicola*, *A. paludosa* and *A. undulata*, measured by Malcolm, Clarke,

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D'Antuono & Swaan (1988), were very similar to the values for the same species in this experiment.

Looking at the mean *in vitro* digestibility of the edible component over the entire period (Figure 12), it can be seen that digestibility ranged from 42% (*A. glauca*) to 60% (*A. nummularia* and *A. paludosa*). Digestibility of edible DM, which also included edible stems, as determined by

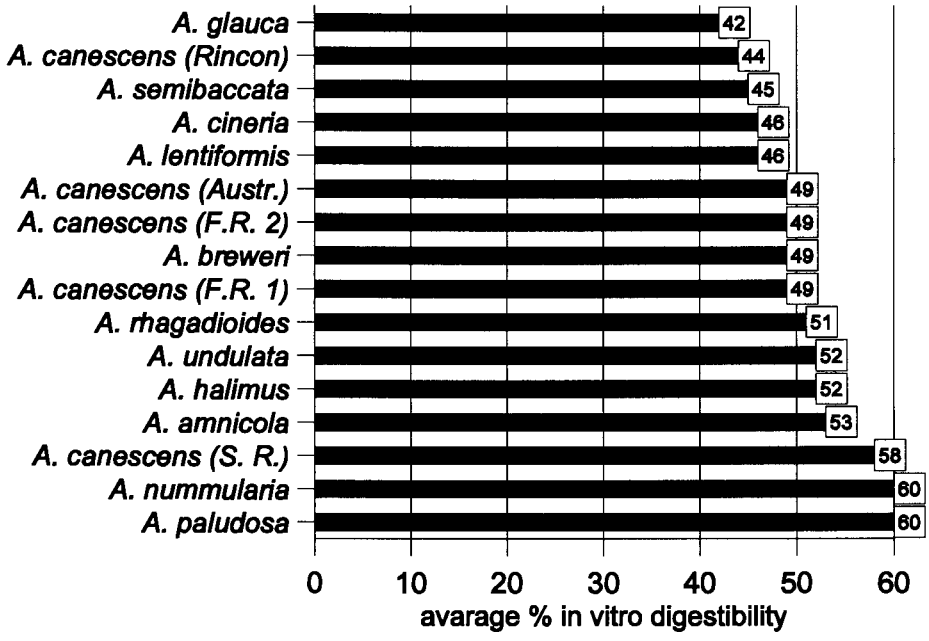

Figure 12 Average total apparent DM *in vitro* digestibility of the edible component of the 16 Atriplex accessions.

Warren, Bunny & Bryant (1990) was more or less the same for *A. undulata* (53%) and *A. amnicola* (57%) in this experiment, but for *A. cinerea* (60%) and *A. lentiformis* (62%) it was much higher than the 46% registered for both in this experiment.

In general leaves had a much higher *in vitro* digestibility than edible stems with *A. nummularia*, *A. paludosa* and *A. canescens* (Santa Rita) having the highest total edible component *in vitro* digestibility. Variation between the three stages might be a result of plant age, but the influence of the different seasons might also have contributed to the difference. Differences between accessions varied a lot and probably expressed the genetic variation both within and between the different species.

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Neutral-detergent fibre (NDF)

According to Van Soest (1983) NDF is the logical replacement for crude fibre. Neutral-detergent fibre represents the sum of lignin, cellulose and hemicellulose, components which have diverse effects upon nutritive value. It is more highly correlated with intake than is any other feed fraction.

The NDF was determined for leaves and edible twigs at three stages and is illustrated in Table 9. Average NDF values ranged from as low as 46% for *A. paludosa* to as high as 62% for *A. canescens* (Rincon). Values for leaves were much lower than stem values, with the leaves of *A. paludosa* (27%)the lowest and those of *A. semibaccata* (44%) and *A. cineria* (44%) the highest. The NDF values for the leaves were, in general, lower in autumn (11 months after planting) than in winter (15 months after planting), which agrees with the results of Colomer & Passera (1990) for *A. cinerea*, *A. nummularia*, *A. rhagadioides* and *A. undulata*.

Table 9 Percentage NDF (DM) of the edible parts, at three stages after planting. Total indicates the percentage NDF of total edible components at each period and was compiled from the proportion of leaves and twigs total edible DM yield

Accessions	8 months	11 months			15 months			Total
	Total	Leaves	Twigs	Total	Leaves	Twigs	Total	mean
(a) *A. amnicola*	51 b,e,f,j,l,m	42	73	62 d,e,h,i,o	38	72	55	56
(b) *A. breweri*	52 a,e,f,j,l,m,o	34	75	59 e,k,n	31	77	63 c,d,e,h,o	58
(c) *A. canescens* (Austr.)	59 d,i	34	79	50	33	81	64 b,d,e,h,o	58
(d) *A. canescens* (F.R. 1)	61 c,i	42	80	62 a,e,h,i,k,n	38	77	63 b,c,e,h,k,o	62
(e) *A. canescens* (F.R. 2)	50 a,b,f,j,l,m,n	42	78	60 a,b,d,h,i,k,n,p	37	75	63 b,c,d,h,i,k,p	58
(f) *A. canescens* (Rincon)	52 a,b,e,j,l,m	32	74	55 g,j	34	77	66 o	58
(g) *A. canescens* (S. R.)	43	41	81	55 f	33	76	50	49
(h) *A. cineria*	56 k,o	44	76	61 a,d,e,i,n	40	75	64 b,c,d,e,o	60
(i) *A. glauca*	59 c,d,o	40	74	62 a,d,e,h,o	33	72	61 e,k,n	61
(j) *A. halimus*	50 a,b,e,f,l,m,n	39	72	54 f	32	67	47 l,m	50
(k) *A. lentiformis*	56 h,o	38	74	59 b,d,e,n,p	33	74	61 e,k,n	59
(l) *A. nummularia*	51 a,b,e,f,j,m,n	39	68	47	35	66	46 j,m	48
(m) *A. paludosa*	52 a,b,e,f,j,l	27	72	42	34	70	45 j,l	46
(n) *A. rhagadioides*	48 e,j,l	41	74	60 b,d,e,h,k,p	36	76	59 i,p	56
(o) *A. semibaccata*	55 b,h,i,k	44	72	63 a,i	42	71	64 b,c,d,f,h	61
(p) *A. undulata*	*nm	43	74	58 e,k,n	40	74	60 e,k,n	59

Superscript: indicates values that **did not** differ significantly ($p<0.05$) from other accessions, in the same column, marked with the same letter

* not measured

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In Figure 13 the average NDF values for the total edible components at all three stages (ages/seasons) are illustrated. From this figure it is clear that *A. paludosa*, *A. nummularia*, *A. canescens* (Santa Rita) and *A. halimus* had the lowest NDF values with *A. canescens* (F.R.1), *A. glauca* and *A. semibaccata* the highest. These results were similar to those of Watson *et al* (1987) for *A. nummularia* and *A. lentiformis*.

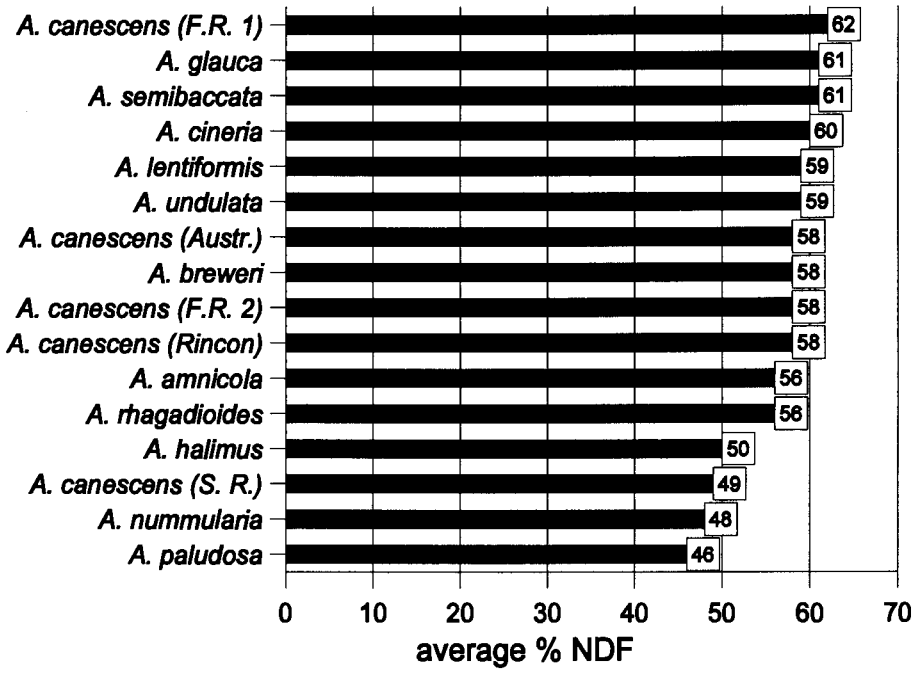

Figure 13 Average NDF of the edible component of each of 16 *Atriplex* accessions.

Crude protein (CP)

Total crude protein production per hectare was discussed earlier in this chapter in Figure11. The CP-content of the different edible components at the three stages is illustrated in Table 10. The leaves had a much higher CP-content than the edible twigs, with the edible twigs of *A. nummularia* significantly higher than the edible twigs of any other accession. There were quite a few other accession with leaf CP-contents similar or higher than that of *A. nummularia*, but the high CP-content in the edible twigs of *A. nummularia* resulted in this accession having the highest average CP-content. Crude protein contents of the leaves were, in general, lower in autumn (11 months after planting) than in winter (15 months after planting), which is similar to the results of Colomer & Passera (1990) for *A. cineria*, *A. nummularia*, *A. rhagadioides* and *A.*

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undulata. In this experiment the opposite was true for the edible twigs which had, in general, a lower CP-content in winter.

Table 10 Percentage CP (DM) of the edible parts, at three stages after planting. Total indicates the percentage CP of total edible component at each stage and is compiled from the proportion of leaves and twigs in the total edible DM production

Accessions	8 months	11 months			15 months			Total
	Total	Leaves	Twigs	Total	Leaves	Twigs	Total	mean
(a) *A. amnicola*	13	15	7	10	18	5	11	11
(b) *A. breweri*	16	17	7	11	21	5	10	12 [o]
(c) *A. canescens* (Austr.)	12	14	6	11	18	4	9	11
(d) *A. canescens* (F.R. 1)	10	15	7	11	18	7	11	11 [g]
(e) *A. canescens* (F.R. 2)	12	13	7	10	13	3	6	10 [g,j,n]
(f) *A. canescens* (Rincon)	12	13	7	10	16	6	9	10 [g,j,n]
(g) *A. canescens* (S. R.)	15	14	6	11	19	5	14	13 [d,e,f,o]
(h) *A. cineria*	15	17	8	12	15	5	8	12
(i) *A. glauca*	9	12	5	7	16	4	7	8 **
(j) *A. halimus*	16	13	6	10	18	5	13	13 [e,f,o]
(k) *A. lentiformis*	12	18	9	13	20	7	11	12 [o]
(l) *A. nummularia*	16	15	12	14	18	10	15	15 ***
(m) *A. paludosa*	15	11	9	10	13	8	11	12 [o]
(n) *A. rhagadioides*	15	14	8	11	21	6	12	13 [e,f,o]
(o) *A. semibaccata*	13	12	7	8	11	6	7	9 [b,g,j,k,m,n,p]
(p) *A. undulata*	*nm	14	7	11	18	8	12	11 [o]

Superscript: indicates values that differed significantly ($p<0.05$) from other accessions, in the same column, marked with the same letter

* not measured

** *Atriplex glauca* differed significantly ($p<0.05$) from all accessions, except from *A. canescens* (F.R. 1), *A. canescens* (F.R. 2), *A. canescens* (Rincon) and *A. semibaccata*

*** *Atriplex nummularia* differed significantly ($p<0.05$) from all accessions, except from *A. canescens* (S.R.), *A. halimus*, *A. rhagadioides* and *A. undulata*

In Figure 14 the average CP content of the total edible components over the three stages is illustrated. In this figure a clear division between accessions is evident. *Atriplex nummularia* has the highest average CP content, at 15% with *A. rhagadioides*, *A. halimus* and *A. canescens* (Santa Rita) second highest, at 13%. These results differ markedly from the mean CP content of edible biomass of 21.7 % and 17.7 % for *A. nummularia* and *A. halimus* respectively reported

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by Correal (1993) whose values are closer to the values for leaves obtained in this experiment. It is, however, difficult to compare these results with results from the literature, as different researchers have different norms for edible biomass. Some researchers like Benjamin *et al* (1992) described it as leaves plus shoot tips without defining the diameter of the shoot tips while others, like Warren *et al* (1990), defined it as stems with a diameter of 5 to 6 mm. Others, like Correal (1993) talk about edible biomass without defining it at all. In this experiment edible twigs were twigs (stems) with a diameter of 4 mm and less. Concerning the huge difference between leaves and twigs for CP contents (Table 10) and NDF values (Table 9) it is clearly understandable that the proportion of twigs in the edible biomass will have a definite influence on the determination of factors influencing nutritive value. Crude protein values for *A. amnicola* (9.4%), *A. cineria*

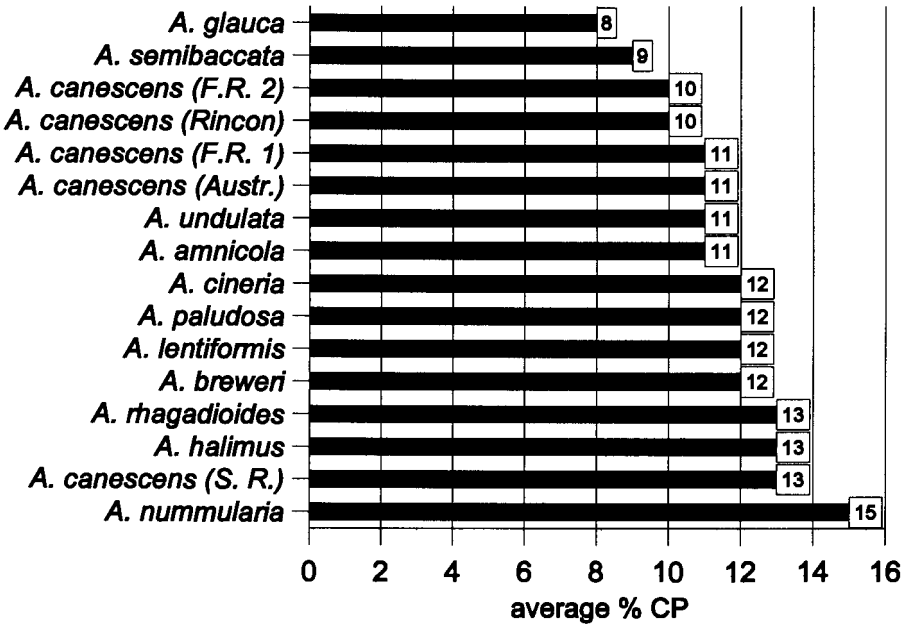

Figure 14 Average total CP of the edible component of the 16 *Atriplex* accessions

(9.0%), *A. lentiformis* (12.4%) and *A. undulata* (10.2%) reported by Warren *et al* (1990), were, however, within 1.5% of the results obtained in this experiment.

According to Atiq-Ur-Rehman, Mackintosh, Fortune and Warren (1994) voluntary intake of diets with CP levels of less than 7% are very low. Colomer and Passera (1990) also stress the

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importance that fodder should have approximately 7% of CP for adequate ruminal activity. From Figure 14 it can be seen that *A. glauca* has the lowest CP-level at only 8%. The voluntary food intake of *A. glauca* would, therefore, be low but acceptable. According to Petersen, Ueckert, Potter & Huston (1987) the CP-levels in four *A. canescens* ecotypes were sufficient to keep ruminants at a good level of nutrition and for body maintenance. These latter results are similar to the results obtained for *A. canescens* in this experiment.

Sodium and ash

Ash gives an indication of the total mineral content, but could be misleading in some fodder trees and shrubs because of high levels of silica or other non-nutritious elements (Van der Merwe & Smith, 1991). Malcolm *et al* (1988) calculated dry matter yields on an ash-free basis, due to the high ash content of *Atriplex* species. According to them expressing the yields on an ash-free basis changes the relativity of the yield differences between species in favour of those with lower ash-contents. According to König (1992) the *in vitro* DM digestibility is an over estimation for leaves and twigs of *Atriplex* spp. due to high levels of ash, Na^+ and K^+ resulting in higher values than *in vitro* OM digestibility.

The ash percentages in this experiment are presented in Table 11. The ash content of twigs was lower than ash content of leaves. Eleven months after planting the ash content of edible twigs was much higher than that of edible twigs 15 months after planting, because of the relatively young and more herbaceous twigs at this early (11 months after planting) stage. At 15 months after planting the ash content of total edible material (biomass) was also much lower than that at the other two stages. This is the result of the stem to leaf ratio, which increased as the plants matured, with the proportion of stems (with lower ash) increasing. This was also reported

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Table 11 Percentage ash (DM) of the edible parts, at three stages after planting. Total indicates the percentage ash of total edible component at each stage and is compiled from the proportion of leaves and twigs in the total edible DM yield

Accessions	8 months	11 months			15 months			Total
	Total	Leaves	Twigs	Total	Leaves	Twigs	Total	mean
(a) *A. amnicola*	22	22	16	18	23	5	14	18
(b) *A. breweri*	23	25	10	16	21	5	9	16 [j,l]
(c) *A. canescens* (Austr.)	18	20	15	18	19	4	9	15 [j,l,n]
(d) *A. canescens* (F.R. 1)	19	25	15	20	23	4	11	17
(e) *A. canescens* (F.R. 2)	24	22	15	18	21	4	10	17
(f) *A. canescens* (Rincon)	22	20	12	16	22	4	9	15 [j,l,n]
(g) *A. canescens* (S. R.)	23	19	15	18	19	4	13	18
(h) *A. cineria*	26	20	17	18	23	6	11	18
(i) *A. glauca*	22	22	13	16	18	6	10	16 [j,l]
(j) *A. halimus*	24	26	15	21	26	7	18	21 [b,c,f,i,k,o,p]
(k) *A. lentiformis*	18	23	12	17	19	6	10	15 [j,l,n]
(l) *A. nummularia*	22	23	17	22	21	7	16	20 [b,c,f,i,k,p]
(m) *A. paludosa*	24	35	20	30	27	9	22	25 **
(n) *A. rhagadioides*	26	23	18	20	22	4	12	19 [c,f,k,p]
(o) *A. semibaccata*	22	24	16	19	24	7	11	17 [k,j]
(p) *A. undulata*	*nm	23	15	19	20	4	11	15 [j,l,n]

Superscript: indicates values that differed significantly ($p<0.05$) from other accessions, in the same column, marked with the same letter

* not measured

** *Atriplex paludosa* differed significantly ($p<0.05$) from all other accessions

by Watson *et al* (1987) for *A. lentiformis* and *A. nummularia*. The results for *A. nummularia*, *A. lentiformis* and *A. canescens* were in agreement with results for the same species by Jacobs & Smit (1978) and Watson *et al* (1987) on normal agricultural soil. While the ash contents found by Malcolm *et al* (1988) for leaves and twigs of *A. amnicola*, *A. paludosa* and *A. undulata* were much higher on a salt effected soil than the ash contents from this experiment, where the soil had very low salt content. Malcolm *et al* (1988) also found that *A. paludosa* had higher ash contents in the leaves and the twigs then any other tested species. From the results of this experiment, on non-saline soil, and the results of Malcolm *et al* (1988), on a salt effected soil, it is clear that ash content of *A. paludosa* is not only a result of soil conditions but also a genetic characteristic of the different species.

Sodium (Na) content of the different edible components of the 16 accessions can be seen in Table 12. Sodium levels for twigs were lower than for leaves, with the leaves of *A. nummularia* the highest at almost 4% and that of *A. canescens* (Santa Rita) the lowest at 0.04%. For the total

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Table 12 Percentage Na in the edible parts, at three stages after planting. Total indicates the percentage Na of total edible component in each period and is compiled from the proportion of leaves and twigs in the total edible DM production

Accessions	8 months	11 months			15 months			Total
	Total	Leaves	Twigs	Total	Leaves	Twigs	Total	mean
(a) *A. amnicola*	2.46	3.12	0.98	1.76	3.26	0.75	2.00	2.07
(b) *A. breweri*	2.38	3.28	1.21	2.02	3.92	0.86	1.75	2.05
(c) *A. canescens* (Austr.)	0.17	0.02	0.00	0.01	0.18	0.11	0.13	0.10 **
(d) *A. canescens* (F.R. 1)	2.34	3.12	0.64	1.83	2.71	0.29	1.18	1.78 [j,m]
(e) *A. canescens* (F.R. 2)	2.30	2.77	0.61	1.65	3.15	0.49	1.36	1.77 [j,m]
(f) *A. canescens* (Rincon)	0.27	0.00	0.03	0.02	0.35	0.00	0.09	0.13 ***
(g) *A. canescens* (S. R.)	0.09	0.01	0.03	0.02	0.04	0.00	0.03	0.05 ****
(h) *A. cineria*	2.12	3.02	1.41	2.15	3.54	1.12	1.90	2.06
(i) *A. glauca*	1.83	2.25	0.51	1.12	2.40	0.69	1.18	1.38 #
(j) *A. halimus*	2.81	3.54	0.93	2.38	3.03	0.41	1.92	2.37 [d,e]
(k) *A. lentiformis*	2.22	2.99	1.19	1.94	3.40	0.95	1.70	1.95
(l) *A. nummularia*	2.55	3.53	1.27	2.88	3.98	0.59	2.73	2.72 ##
(m) *A. paludosa*	2.34	2.83	1.28	2.31	3.48	0.50	2.54	2.40 [d,e]
(n) *A. rhagadioides*	2.09	3.18	1.32	2.09	3.61	0.68	1.92	2.03
(o) *A. semibaccata*	2.48	2.33	0.54	1.09	2.70	0.27	0.79	1.45 ###
(p) *A. undulata*	*nm	3.38	0.61	2.09	3.82	0.37	1.81	1.95

Superscript: indicates values that differed significantly ($p<0.05$) from other accessions, in the same column, marked with the same letter

* not measured

** *Atriplex canescens (Austr.)* differed significantly ($p<0.05$) from all other accessions, except *A. canescens* (S.R.), and *A. canescens* (Rincon)

*** *Atriplex canescens* (Rincon) differed significantly ($p<0.05$) from all other accessions, except *A. canescens (Austr.)* and *A. canescens* (S.R.)

**** *Atriplex canescens* (S.R.) differed significantly ($p<0.05$) from all other accessions, except *A. canescens* (Austr.) and *A. canescens* (Rincon)

Atriplex glauca differed significantly ($p<0.05$) from all other accessions, except *A. canescens* (F.R. 1), *A. canescens* (F.R. 2) and *A. semibaccata*

Atriplex nummularia differed significantly ($p<0.05$) from all other accessions, except *A. halimus* and *A. paludosa*

Atriplex semibaccata differed significantly ($p<0.05$) from all other accessions, except *A. canescens* (F.R.1), *A. canescens* (F.R. 2) and *A. glauca*

edible biomass the Na-content was the lowest for the *A. canescens* accessions and for *A. semibaccata* and the highest for *A. nummularia* and *A. paludosa*. Jacobs & Smith (1978) found the Na contents of *A. nummularia* and *A. canescens* to be 4.2% and 1.4% respectively, which is in agreement with the results in Table 12. According to Watson & O'Leary (1993) *A.*

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canescens, *A. nummularia* and *A. undulata* can reach Na levels of 3.4%, 7.2% and 7.4%, respectively when irrigated with saline water. The high ash and Na values of *A. halimus*, *A. nummularia* and *A. paludosa*, in comparison with the other accessions, might influence the palatability of these species. Species such as *A. semibaccata*, with lower ash and Na levels, might in this context have a higher palatability.

Percentage organic matter (OM)

Organic matter (OM) content was determined as the difference between the total DM and ash. These results are presented in Table 13. The relatively low OM values of *A. halimus*, *A. nummularia* and *A. paludosa*, indicates their high ash content which might influence the palatability of these species. The OM values for leaves stayed more or less constant over time, while the OM levels of the twigs increased from 11 months after planting to 15 months after planting.

Table 13 Percentage OM content of edible material, at three stages after planting. Total indicates the percentage OM of total edible component at each period and is compiled from the proportion of leaves and twigs in the total edible DM production

Accessions	8 months	11 months			15 months			Total
	Total	Leaves	Twigs	Total	Leaves	Twigs	Total	mean
(a) *A. amnicola*	78	78	84	82	77	95	86	82
(b) *A. breweri*	77	75	90	84	79	95	91	84 [j,l]
(c) *A. canescens* (Austr.)	82	80	85	82	81	96	91	85 [j,l,n]
(d) *A. canescens* (F.R. 1)	81	75	85	80	77	96	89	83 [j]
(e) *A. canescens* (F.R. 2)	76	78	86	82	79	96	90	83 [j]
(f) *A. canescens* (Rincon)	78	80	88	84	78	96	92	85 [j,l,n]
(g) *A. canescens* (S. R.)	77	81	85	82	81	96	87	82
(h) *A. cineria*	74	80	83	82	77	94	89	82
(i) *A. glauca*	78	78	87	84	82	94	90	84 [j,l,n]
(j) *A. halimus*	76	74	85	79	74	93	82	79 [b,c,d,e,f,i,k,o]
(k) *A. lentiformis*	82	77	88	83	81	94	90	85 [j,l,n]
(l) *A. nummularia*	78	77	83	78	79	93	84	80 [b,c,f,i,k]
(m) *A. paludosa*	76	65	80	70	73	91	79	75 **
(n) *A. rhagadioides*	74	77	82	80	78	96	88	81 [c,f,i,k]
(o) *A. semibaccata*	78	76	84	81	76	93	89	83 [j]
(p) *A. undulata*	*nm	77	85	81	80	96	89	83

Superscript: indicates values that differed significantly ($p<0.05$) from other accessions, in the same column, marked with the same letter

* not measured

** *Atriplex paludosa* differed significantly ($p<0.05$) from all other accessions

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CONCLUSIONS AND RECOMMENDATIONS

From the results it was notable that big differences between the 16 different accessions, were evident with some showing good potential as fodder crops. To compare all the accessions, Table 14 was compiled from the mean values of all the parameters, with the exception of volume, where the last measured volumes were used. The accessions are arranged from those with the highest potential as a fodder crop (*A. nummularia*) to those with the lowest potential as a fodder crop (*A. canescens*, Rincon).

Table 14 Chemical composition, *in vitro* dry matter digestibility (IVDMD), edible DM and CP production and volumes of 16 *Atriplex* accessions. Accessions are arranged from those with the highest potential to those with the lowest potential as fodder crops.

Accessions	Volume	DM edible production	CP	CP	IVDMD	NDF	Ash	Na
	m^3	$t.ha^{-1}$	$kg.ha^{-1}$	% (DM)	% (DM)	% (DM)	% (DM)	% (DM)
A. nummularia	3.28	3.98	596	15.1	60	48	20	2.72
A. canescens (S. R.)	3.92	4.02	550	13.2	58	49	18	0.05
A. rhagadioides	2.70	5.78	718	12.9	51	56	19	2.03
A. amnicola	2.80	5.24	602	11.4	53	56	18	2.07
A. paludosa	0.65	2.78	312	12.0	60	46	25	2.40
A. halimus	2.55	2.81	356	12.9	52	50	21	2.37
A. breweri	2.50	3.50	336	12.2	49	58	16	2.05
A. canescens (Austr.)	2.47	2.14	190	10.8	49	58	15	0.10
A. undulata	0.48	1.34	162	11.4	52	59	15	1.95
A. cineria	2.71	3.74	303	11.8	46	60	18	2.06
A. lentiformis	1.51	1.34	148	12.0	46	59	15	1.95
A. canescens (F.R. 1)	1.84	2.37	258	10.5	49	62	16	1.78
A. canescens (F.R. 2)	1.09	1.76	113	9.7	49	58	17	1.77
A. semibaccata	1.48	5.17	370	9.3	45	61	17	1.45
A. glauca	2.22	5.29	391	8.0	42	61	16	1.38
A. canescens (Rincon)	1.18	0.71	61	10.0	44	58	15	0.13

Atriplex nummularia, *A. canescens* (Santa Rita), *A. rhagadioides* and *A. amnicola* all have a very good potential as fodder crops. The IVDMD of *A. amnicola* and *A. rhagadioides* were a bit low, but their high CP-production count in their favour. Although their CP content is not as high as that of *A. nummularia* and *A. canescens* (Santa Rita), they produced a much higher proportion of edible twigs (Table 7) because of their more herbaceous nature, compared with the more woody nature of the latter. One concern with respect to these four accessions is their relatively

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high ash content which might influence their palatability. In contrast the sodium (Na) content of *A. canescens* (Santa Rita) was much lower than the others and might favour its palatability. The palatability of all accessions will be discussed in more detail in another chapter.

The next accession on the list is *A. paludosa*. Although the edible DM and CP production of this accession is not that high, it produced a higher percentage of leaves (53%) than any other accession (Table 7). Another factor is the size of this plant at only 0.65 m^3, it was the second smallest and the espacement used in this trial for all accessions of 1m x 3m might be adjusted for such a species to 1m x 1.5m. This might double the edible DM and CP production per hectare. Although the NDF value of *A. paludosa* was the lowest (46%), due to the high percentage of leaves in the edible DM, its ash content was the highest (25%) and this might have a negative influence on palatability. The high IVDMD of this accession might also be misleading, as Benjamin *et al* (1995) mentioned that high *in vitro* DM digestibilities of *Atriplex* spp. might be misleading due to the high concentrations of soluble salts (forms part of total ash) in their leaves.

Atriplex halimus had a high CP content of 12.9%, but had a relatively low IVDMD of 52% and a high ash content of 21%, which might influence its palatability negatively. *Atriplex breweri* and *A. canescens* (Australia) are another two promising accessions but their low IVDMD (49%) and high NDF value (58%) might be detrimental to their value as fodder crops.

For some reason *A. undulata* was difficult to establish in the experimental plot. All seedlings in the first planting died and had to be replanted three weeks later. The seedling vigour might have been poor at that stage. After re-establishment, the plants grew well but always lagged behind the other accessions. *Atriplex undulata* had an acceptable CP content of 11.4% (Table 14), but the high NDF value of 59% is of concern. The relatively small size of plants in this accession (0.48 m^3), also raise the possibility of planting it at a higher density.

Atriplex cineria and *A. lentiformis* both have a high CP content (≈12%), but also a very low IVDMD and high NDF values of 46% and 60% respectively, which mitigate against them being considered as good fodder crops.

Atriplex canescens (F.R.1 and F.R.2) are both low in CP (10.5% and 9.7%) and high in fibre (NDF, 58%). These plants are also smaller than the other *A. canescens* accessions and are also more woody. Their value as fodder crops is not high.

Atriplex semibaccata (creeping growth form) and *A. glauca* (prostrate to creeping growth form) are two species which are probably better for erosion control and reclamation of degraded land,

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According to Malcolm *et al* (1988), major differences in plant size (*Atriplex* spp.) could lead to different spacing requirements. In this study only one spacing was used for all the accessions and variation in size between the different accessions did occur. It is suggested that in future an optimum spacing should be determined for each accession for maximum DM production per hectare. Espacement trials were conducted in the past, but suitable spacing for specific species, environmental conditions and/or management practices should still be determined. Another important point is the cost of establishing *Atriplex* plantations. According to Condon, Sippel & Alchin (1991), the cost of planting a hectare of *A. nummularia* can range from 500 to 1000 Australian $ depending on planting density, while planting density is influenced by annual precipitation or irrigation.

The IVDMD and CP content of *Atriplex* spp. might be misleading. According to Benjamin *et al* (1992) the IVDMD of *Atriplex* spp. can be misleading because of the high content of soluble salts in the ash, which contain no energy for animal production. *In vitro* OM digestibility will give lower but more reliable results. Concerning the CP content, which was calculated as %N multiplied by 6.25, these values can also be misleading. According to Wilson (1977) N digestibility of *A. nummularia* is 93% and according to Warren *et al* (1990) the N digestibility of *A. amnicola*, *A. undulata*, *A. lentiformis* and *A. cineria* ranged between 70% to 81% .
It is recommended that the four species identified as best for fodder production, *A. nummularia*, *A. canescens* (Santa Rita), *A. rhagadioides* and *A. amnicola* should be further tested and compared in larger trials. The species identified as those with the lowest value as fodder crops, *A. canescens* (Rincon), *A. glauca* (prostrate to creeping growth form) and *A. semibaccata* (creeping growth form) should be incorporated in trials concerning rehabilitation of degraded land and soil erosion control.

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than as fodder crops. Both these species demonstrated the ability to form roots where stems touched the soil and also to multiply by seedlings emerging in the vicinity of the mother plant. Their growth form and habit of producing seedlings, therefore make these accessions suitable for erosion control and reclamation of degraded land. Their value as fodder crops is low due to low CP levels, low IVDMD and high NDF values. On the other hand their high proportion of edible DM and low ash contents (higher palatability) does not exclude further research on these accessions as fodder crops.

Atriplex canescens (Rincon) is a woody plant that does not grow very large and has a very low leaf production. Although its CP content is 10%, it produced only 61 kg. edible CP per hectare in comparison with the 113 $kg.ha^{-1}$ of *A. canescens* (F.R.2) which was the second poorest in this respect.

From this study it is evident that genetic variation within a species should be a matter of concern when working with *Atriplex* species. This was clearly illustrated by the big differences between the five *A. canescens* accessions. Another proof is the large standard deviations as was indicated in Tables 5, 6 and 7. *Atriplex amnicola*, which is the new name for *A. rhagadioides*, as mentioned in the introduction, differed from *A. rhagadioides* and the one can almost be described as a subspecies of the other. The same applies to *A. breweri*, which is described in the literature as a subspecies of *A. lentiformis*. When looking at these plants they look very similar, but comparing them in this study showed clear differences between them. It is, therefore, suggested that in future more time should be spent on selection of suitable ecotypes within species. Plots containing single species should be planted. These individual plants (all from one species) should be screened for individuals with a specific set of criteria. Selections should be made and the selections multiplied.

According to Petersen *et al* (1987) differences in nutritive values may be associated with differential retention of leaves in winter among individual plants and ecotypes. This was something not taken into account in this study. The measurements made at 15 months after planting, were made in winter. Leaf-loss did occur at different rates with some of the species but was unfortunately, not monitored. This is an important characteristic that might have a large influence on differences between accessions and between seasons, as more nutrients are allocated to the leaves and these form the major part of the edible biomass.

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CHAPTER 3

ADAPTATION AND PRODUCTIVITY OF *ATRIPLEX SPP* UNDER DIFFERENT ECOLOGICAL CONDITIONS

ABSTRACT

Because of regular droughts and relatively poor soil conditions, semi-arid and arid areas of South Africa have extremely low carrying capacities. Many *Atriplex* spp. are well adapted to these conditions and together with their high nutritional value they can serve as a fodderbank for both communal and commercial farmers. The following 12 species were evaluated: *Atriplex amnicola*, *A. breweri*, *A. canescens*, *A. cinerea*, *A. glauca*, *A. halimus*, *A. lentiformis*, *A. nummularia*, *A. paludosa*, *A. rhagadioides*, *A. semibaccata* and *A. undulata*. The different sites were situated in the Free State, Gauteng and Northern Cape provinces. Sites differ with respect to mean annual rainfall, mean annual temperature and soil physical- and chemical conditions. Adaptation (survival), nutritive quality (*in vitro* digestibility, neutral detergent fibre, sodium and crude protein content, % ash.) and growth were recorded. Species differed, but *Atriplex amnicola, A. canescens* (Santa Rita), *A. halimus* and *A. nummularia* were the best adapted. Such species may be incorporated into production systems, involving farmers and extension officers in a farm systems research and extension programme, which include both "on farm" and "on station" work.

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INTRODUCTION

Almost two thirds of South Africa is semi arid to arid. Sixty four percent of the area receives an annual rainfall of 500mm or less (Le Houérou, 1994). The main agricultural activity in these areas is stock farming and land users are largely dependent on the range (Anon, 1994). Overgrazing has aggravated the situation and resulted in extremely low carrying capacities. The balance between the demands of livestock and the grazing capacity in such areas is very precarious. Some areas have undergone processes of severe degra ation or have been subjected to desertification. According to Le Houérou (1992) a plantation of *Atriplex* spp. appears to be one of the best ways to rehabilitate desertified areas and restore them to production.

Although not all areas are overgrazed, during droughts most land users struggle to keep their livestock alive. During such droughts an alternative fodderbank, to be used for livestock and to protect the range from overgrazing could be valuable. Bosch (1979) emphasized the importance of withdrawing grazing animals from the range directly after the first rainfall at the end of a period of drought. If the animals are withdrawn from the range to a camp with planted fodder shrubs, like *Atriplex*, new seedlings will have time to establish and the species composition will improve rather than decline, as would have been the case if the animals were left on the range to continue grazing after the rainfall.

There is, therefore, a need for alternative crops, which are well adapted and also have a high grazing value. Such crops must be palatable, nutritive, and must recover well after grazing. The genus *Atriplex* has a great number of species distributed in the semi-arid and arid zones of the world (Osmond, Björkman & Anderson, 1980). Native stands of *Atriplex* can produce yields of 400-2000 kgDM/ha/year. Some recorded nutritive values for different *Atriplex* spp. are an *in vitro* digestibility of leaves ranging from 74 to 78%, crude protein content of 10 to 20% and neutral detergent fibre of 32 to 45% (Colomer & Passera, 1990). Some *Atriplex* spp. are well adapted to local conditions and, together with their high nutritional value, they can serve as a fodderbank for both communal and commercial farmers.

The objective of this study was to test the adaptability and nutritive values of several *Atriplex* spp. under different ecological conditions in the semi-arid and arid areas of South Africa.

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MATERIALS AND METHODS

The twelve species that were studied, together with some cultivars and selections of *A. canescens*, are listed in Table 1. The origin of each species is also listed as described by Osmond *et al* (1980). Not all the species were planted at all the locations as seedlings of all the species were not available when the experimental plots were established at the different locations.

Table 1 Names and origin of *Atriplex* accessions evaluated

Accessions	Origin
A. amnicola Paul G. Wilson	Western Australia
A. canescens (Pursh.) (Australia)	Australia
A. canescens (Pursh.) (Rincon)	North America
A. canescens (Pursh.) (Santa Rita)	North America
A. canescens (Pursh.) (Field Reserve 1)	North America
A. canescens (Pursh.) Field Reserve 2)	North America
A. cinerea Poir.	Australia, Europe, South Africa
A. glauca L.	Mediterranean, South Africa
A. halimus L.	Asia, Mediterranean
A. lentiformis ssp. *breweri* (Wats.)Hall&Clem.	North America
A. lentiformis (Torr.) Wats. ssp. *lentiformis*	North America
A. nummularia L.	Australia
A. paludosa R. Br.	Australia
A. rhagadioides F. Muell.	Australia
A. semibaccata R. Br.	The Americas, Europe, Australia & South Africa
A. undulata D. Dietr.	South America

Experimental sites were in the Free State Province (Bloemfontein), Gauteng Province (Pretoria) and Northern Cape Province (Kathu, Mier, Pofadder). A minimum of two replicates were planted at each location with five plants of each species per replicate, at each location. The spacing was 1.5m in the row and 5m between rows (1333 plants per hectare) for all locations except Pretoria, where it was 1m in the row and 3m between rows (3333 plants per hectare).

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The mean annual rainfall, mean annual temperatures and soil physical- and chemical conditions are illustrated in Tables 1 and 2.

Table 2 Climatic parameters of each site. Mean annual figures for each location

Site	Rainfall (mm)	Temperature(°C)	
		min	max
Bloemfontein	549	7.5	24.3
Pretoria	650	11	25.0
Kathu	335	12.1	25.3
Mier	213	11	29.4
Pofadder	117	11.4	25.8

From Table 2 it can be seen that Pretoria and Bloemfontein had mean annual rainfall figures of higher than 500mm while the other locations were lower than 350mm and thus much drier. Concerning the mean maximum temperatures, Mier was the hottest, at ≈30°C, with the other locations averaging ≈25°C. Bloemfontein was the coldest site.

Table 3 Chemical and physical soil parameters of each site

Site	Clay (%)	pH(H_2O)	Ca (ppm)	Mg (ppm)	P (Bl) (ppm)	K (ppm)	Na (ppm)
Bloemfontein	7	7.1	725	264	105	342	31
Pretoria	38	6.6	861	201	34	248	34
Kathu	<10	8.5	4212	266	23	233	76
Mier	< 5	6.3	188	71	11	95	5
Pofadder	<10	8.6	2213	291	70	1300	672

From Table 3 it is evident that the soil at Pretoria had a higher clay content and that the soil at Kathu and Pofadder were more alkaline than the other locations, with Na content of the Pofadder soil being particularly notable. The sandy soil at Mier was much lower in nutrient content than the other locations and had the lowest pH.

The altitude of each site is: Bloemfontein - 1351m, Pretoria - 1360m, Kathu - 1187m, Mier - 879m and Pofadder - 989m.

At each location survival and growth were recorded. Growth was determined by measuring

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height and width at different stages after planting. From these measurements the shrub volumes was determine as the volume of a cylinder. The formula for the volume of a cylinder is: v(cylinder) = $\pi r^2 h$, where r = half the diameter (half of the plant width) and h = height (plant height) and $\pi = 3.143$.

Chemical analyses to determine nutritive quality included: *in vitro* digestibility, using the Tilley and Terrey technique as adapted by van der Merwe (Van Der Merwe & Smith, 1991); neutral detergent fibre (NDF), using the Dosi Fiber system with Robbertson and Van Soest method (Roberston & Van Soest, 1981); sodium (Na), using a wet calcination and sulphur acid technique; and crude protein (CP)/N - content, using the Kjeldahl technique (AOAC, 1984). Other measurements were dry matter (DM), organic matter (OM) and ash contents that were determined by oven drying. Dry matter was determined by weighing two grams of the sample and drying at 100°C for 24 hours or until constant weight. Ash was determined by incinerating putting the weighed samples in an oven at 600°C for 4 hours. Organic matter was determined as the difference between DM and ash.

RESULTS AND DISCUSSION

Not all species were planted at all sites (dnp - did not plant) and of the species planted not all were measured (nm - not measured).

Survival and growth

Species did not perform equally well at all sites. This conclusion is based on survival (Table 4) and plant vigour as reflected by plant volume (Table 5). All five *A. canescens* accessions, *A. glauca*, *A. halimus* and *A. nummularia* showed average (for all locations) survival rates of 87% and higher and might thus be well adapted to a wide range of ecologically condition in the semi-arid and arid areas of South Africa. *Atriplex cinerea* and *A. paludosa* showed survival rates (average for all locations) of 46% and 25% respectively, which was rather low. The average survival rate for all the plants, at each location, was low at Bloemfontein, at 41%, while at Mier it was high at 87%. This can be the result of a combination of many ecological, climatological and management factors. The co-worker at Mier was, for example, very active in ensuring the

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survival of all the accessions on his farm and irrigated the plants when ever the soil was dry and also controlled weeds effectively in the experimental plot. These management practices resulted in the high average survival rate at Mier.

Table 4 Percentage survival at each site, as well as the average (avg) over all sites. Plants were one year old

Accessions	Bloemfontein	Pretoria	Kathu	Mier	Pofadder	avg
A. amnicola	0	95	80	90	0	53
A. breweri	30	65	90	100	80	73
A. canescens (Aus)	*dnp	90	dnp	100	90	93
A. canescens (F.R.1)	100	80	100	100	100	96
A. canescens (F.R.2)	dnp	80	90	dnp	90	87
A. canescens (Rin)	dnp	90	dnp	100	90	93
A. canescens (SR)	70	85	100	100	100	91
A. cinerea	0	80	60	40	0	36
A. glauca	100	100	80	100	100	96
A. halimus	100	100	90	100	90	96
A. lentiformis	20	65	100	100	100	77
A. nummularia	80	100	100	100	100	96
A. paludosa	0	90	dnp	10	0	25
A. rhagadioides	0	95	70	100	0	53
A. semibaccata	20	85	60	100	80	69
A. undulata	10	70	50	70	80	56
Average	41	86	82	87	69	74

*did not plant

With respect to the relative plant volume (Table 5), Pretoria, Mier and Bloemfontein, had higher relative plant volumes than Kathu and Pofadder. This might be due to the higher annual rainfall in Pretoria and Bloemfontein and the good management of the experimental plot at Mier. In Kathu and Pofadder the mean annual rainfall of 335mm and 117mm respectively is very low and probably have contributed to weak development of plants and thus low relative volumes. According to Le Houérou (1992) most *Atriplex* spp. can develop under cultivation in the arid zone where the mean annual rainfall is 200mm and higher. If the mean annual rainfall drops below 200mm, additional water from runoff or irrigation, or the presence of a water table is necessary for good production.

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Table 5 Relative volume (m^3) of plants at different sites.

Accessions	12 months after planting				18 months after planting	
	Pretoria	Kathu	Mier	Pofadder	Bloemfontein	Pretoria
A. amnicola	1.78	0.16	1.60	0.00	0.00	2.94
A. breweri	1.60	0.03	1.28	0.29	0.57	1.92
A. canescens (Aus)	1.53	*dnp	0.42	0.13	dnp	1.96
A. canescens (F.R.1)	1.02	0.04	0.30	0.05	0.79	1.44
A. canescens (F.R.2)	1.68	0.07	dnp	0.06	dnp	0.98
A. canescens (Rin)	0.77	dnp	0.24	0.06	dnp	0.33
A. canescens (SR)	2.91	0.13	1.38	0.35	2.81	3.89
A. cinerea	1.63	0.20	0.47	0.00	0.00	2.34
A. glauca	1.22	0.05	0.12	0.41	0.60	1.05
A. halimus	3.17	0.08	1.10	0.21	0.55	3.34
A. lentiformis	1.06	0.11	1.67	1.34	0.04	1.06
A. nummularia	1.17	0.11	0.65	0.10	2.59	3.08
A. paludosa	0.34	dnp	0.09	0.00	0.00	0.27
A. rhagadioides	1.80	0.09	0.21	0.00	0.00	0.74
A. semibaccata	1.30	0.07	0.18	0.08	0.08	1.39
A. undulata	0.65	0.05	0.37	0.06	0.22	0.42

*did not plant

Fibre, crude protein and *in vitro* DM digestibility

With respect to quality parameters, data on neutral detergent fibre (NDF) content, CP content and *in vitro* DM digestibility are presented in Tables 6, 7 and 8. These data are not available for Bloemfontein as no plant material was collected from this location. From the other four locations only edible samples were taken which included young twigs and leaves and no woody material (thick twigs). Edible material was hand picked from four or more plants of each accession and mixed to form one representative sample of all the individual plants of the same accession for the total experimental plot. It is not desirable to compare the different locations according to these measurements as the plant material was collected in different seasons. Although the plants at all four locations were approximately one year old, they were planted and sampled at different seasons. Colomer & Passera (1990) reported that the chemical composition of *Atriplex* spp. varied substantially between seasons. The samples were also taken by different workers and unfortunately there was no control over the twig diameter as to whether it was edible or not. Only at Pretoria were all twigs with a diameter of 4mm and less taken as edible. For the other locations the edible material consisted of mainly younger twigs with diameters of ≈3mm

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and less.

Neutral detergent fibre varied from 32 to 69 % and the results are presented in Table 6. Pretoria had the highest average of 57% which might be the result of the thicker twigs that were allowed in the samples as part of the edible component. The lowest average NDF values were at Kathu (36%) and Mier (38%). In Pretoria *A. semibaccata* had the highest NDF value of 69% with *A. nummularia* the lowest at 53%. In Kathu *A. amnicola* had the highest NDF value of 40% with *A. lentiformis* the lowest at 33%. At Mier *A. breweri* had the highest NDF value of 44% with *A. halimus* the lowest at 32%, while in Pofadder *A. canescens* (Aus) and *A. canescens* (F.R.2) had the highest NDF values, at 51%, with *A. semibaccata* the lowest, at 36%. None of the accessions or locations produced NDF values unacceptable to animals.

Table 6 Percentage Neutral Detergent Fibre (NDF) of the edible component. Plants were one year old

Accessions	%NDF (DM)			
	Pretoria	Kathu	Mier	Pofadder
	autumn	summer	spring	summer
A. amnicola	68	40	37	nm
A. breweri	65	*nm	44	49
A. canescens (Aus)	56	**dnp	nm	51
A. canescens (F.R.1)	68	36	nm	47
A. canescens (F.R.2)	66	nm	dnp	51
A. canescens (Rin)	60	dnp	nm	47
A. canescens (SR)	61	34	34	46
A. cinerea	68	nm	nm	nm
A. glauca	68	nm	nm	46
A. halimus	59	34	32	41
A. lentiformis	65	33	38	45
A. nummularia	53	36	41	44
A. paludosa	46	dnp	nm	nm
A. rhagadioides	66	38	nm	nm
A. semibaccata	69	nm	nm	36
A. undulata	63	nm	nm	40
Average	57	36	38	49

* not measured **did not plant

Crude protein (CP) content varied from 7 to 21% and the results are presented in Table7. The CP content was the lowest at Pretoria (11%) and the highest at Mier (18%) and Kathu (17%) with Pofadder(15%) intermediate. The CP values for all the plants at Pretoria were relatively low in

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comparison with the other locations, but this might again be the result of the thicker twigs in the samples. In Pretoria *A. nummularia* had the highest CP content of 14% with *A. glauca* the lowest at 7%. At Kathu *A. canescens* (F.R.1) had the highest CP content of 20% with *A. lentiformis* the lowest at 15%. At Mier *A. halimus* had the highest CP content of 21% with *A. lentiformis* the lowest at 15%, while in Pofadder *A. halimus* had the highest CP content of 19% with *A. breweri* and *A. canescens* (F.R.1) the lowest, both at 12%. All the CP values are, however, relatively high and acceptable for utilization by animals.

Table 7 Percentage Crude Protein (CP) of the edible component. Plants were one year old

Accessions	%CP (DM)			
	Pretoria	Kathu	Mier	Pofadder
	autumn	summer	spring	summer
A. amnicola	10	18	20	nm
A. breweri	11	*nm	16	12
A. canescens (Aus)	11	**dnp	nm	14
A. canescens (F.R.1)	11	20	nm	12
A. canescens (F.R.2)	10	nm	dnp	16
A. canescens (Rin)	10	dnp	nm	13
A. canescens (SR)	11	17	18	15
A. cinerea	12	nm	nm	nm
A. glauca	7	nm	nm	14
A. halimus	10	17	21	19
A. lentiformis	13	15	15	16
A. nummularia	14	16	16	14
A. paludosa	10	dnp	nm	nm
A. rhagadioides	11	16	nm	nm
A. semibaccata	8	nm	nm	18
A. undulata	11	nm	nm	17
Average	11	17	18	15

* not measured **did not plant

The *in vitro* DM digestibilities varied from 34 to 72 % and are presented in Table 8. The average *in vitro* DM digestibility in Pretoria was the lowest at 41% with Mier and Kathu the highest at 69% and Pofadder intermediate at 56%. The low values for Pretoria might yet again be the result of the higher fibre content due to thicker twigs in the samples. In Pretoria *A. nummularia* had the highest *in vitro* DM digestibility of 54% with *A. amnicola* the lowest on 34%. At Pofadder *A. semibaccata* had the highest *in vitro* DM digestibility of 64% with *A. glauca* the lowest on

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43%. At the other two locations there were not big differences between the accessions tested. All the *in vitro* DM digestibility values were, however, acceptable for utilization by animals.

Table 8 Percentage *in vitro* DM digestibility of the edible component. Plants were one year old

Accessions	% DM *in vitro* digestibility			
	Pretoria	Kathu	Mier	Pofadder
	autumn	summer	spring	summer
A. amnicola	34	66	72	nm
A. breweri	39	*nm	64	49
A. canescens (Aus)	43	**dnp	nm	56
A. canescens (F.R.1)	35	70	nm	47
A. canescens (F.R.2)	37	nm	dnp	63
A. canescens (Rin)	40	dnp	nm	46
A. canescens (SR)	49	71	73	58
A. cinerea	41	nm	nm	nm
A. glauca	35	nm	nm	43
A. halimus	43	68	70	61
A. lentiformis	41	72	69	63
A. nummularia	54	72	69	57
A. paludosa	53	dnp	nm	nm
A. rhagadioides	38	66	nm	nm
A. semibaccata	35	nm	nm	64
A. undulata	43	nm	nm	60
Average	41	69	69	56

* not measured **did not plant

From these data it is evident that the quality of the edible portions of such species will more than satisfy the maintenance requirements of livestock in most seasons. Initially livestock will select a higher quality diet but as material becomes more limiting, the quality selected, will probably decline.

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Sodium, ash and OM

The Na content of *Atriplex* (see Table 9) may influence palatability. According to the soil analyses (see Table 3), Mier's soil had the lowest amount of Na at only 5 ppm. However, Mier had the highest average percentage of Na of all sampled plants at 2.60%. This might be due to the saline water with which the plants were irrigated by the farmer. It is known that the water on that farm is too saline for human consumption.

According to Ibrahim (1981) *Atriplex* spp. can reach Na levels of up to 8% when growing on saline soils. From Table 9 it is evident that the Na levels of 0.01% to 3.50% of the DM is low enough to be acceptable as fodder.

Table 9 Percentage Sodium (Na) of the edible component. Plants were one year old

Accessions	%Na(DM)			
	Pretoria	Kathu	Mier	Pofadder
	autumn	summer	spring	summer
A. amnicola	1.76	2.67	2.42	nm
A. breweri	2.02	*nm	3.04	1.81
A. canescens (Aus)	0.01	**dnp	nm	1.63
A. canescens (F.R.1)	1.83	2.23	nm	0.13
A. canescens (F.R.2)	1.65	nm	dnp	1.66
A. canescens (Rin)	0.02	dnp	nm	0.39
A. canescens (SR)	0.02	0.08	0.28	0.10
A. cinerea	2.15	nm	nm	nm
A. glauca	1.12	nm	nm	2.05
A. halimus	2.38	2.83	3.29	2.26
A. lentiformis	1.94	2.66	3.25	2.12
A. nummularia	2.88	3.50	3.34	1.98
A. paludosa	2.31	dnp	nm	nm
A. rhagadioides	2.09	3.11	nm	nm
A. semibaccata	1.09	nm	nm	2.44
A. undulata	2.09	nm	nm	2.45
Average	1.59	2.44	2.60	1.59

* not measured **did not plant

It is interesting to note that three of the five *A. canescens* accessions had relatively low Na levels, while the Na levels of *A. breweri*, *A. halimus*, *A. lentiformis* and *A. nummularia* were higher than the other accessions. The Na content differed between sites and between species within sites, indicating soil and genotype effects, which deserve more intensive investigation.

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The percentage ash is presented in Table 10 and ranged from 16 to 30%. The averages of the four sites varied from 19% to 23%. From Table 3 it can be seen that Kathu had a Ca content of more than double that of the other locations and from Table 10 that the plants from Kathu had the highest ash content of 23%. From the soil chemical analysis (Table 3) it might be expected that Pofadder would have had a higher ash content than the other locations, but that was not the case. The soil sample might have contained something like animal manure which influenced the chemical analysis. Watson (1990) reported ash contents of 16 to 37% for *Atriplex* spp. irrigated with saline water, that were still acceptable for utilization by animals.

Table 10 Percentage ash of the edible component. Plants were one year old

Accessions	%Ash(DM)			
	Pretoria	Kathu	Mier	Pofadder
	autumn	summer	spring	summer
A. amnicola	18	19	22	nm
A. breweri	16	*nm	18	20
A. canescens (Aus)	18	**dnp	nm	18
A. canescens (F.R.1)	20	22	nm	20
A. canescens (F.R.2)	18	nm	dnp	18
A. canescens (Rin)	16	dnp	nm	18
A. canescens (SR)	18	20	17	18
A. cinerea	19	nm	nm	nm
A. glauca	16	nm	nm	21
A. halimus	21	26	25	26
A. lentiformis	17	25	20	17
A. nummularia	22	24	19	22
A. paludosa	30	dnp	nm	nm
A. rhagadioides	20	22	nm	nm
A. semibaccata	19	nm	nm	29
A. undulata	19	nm	nm	26
Average	19	23	20	21

* not measured **did not plant

Organic matter (OM) content was determined as the difference between the total DM and ash. These results are presented in Table 11. The OM values of lower than 70% for *A. halimus* and *A. nummularia*, indicates their relatively high ash contents, which might influence the palatability of these species.

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Table 11 Percentage organic matter (OM) of the edible component. Plants were one year old

Accessions	%OM (DM)			
	Pretoria	Kathu	Mier	Pofadder
	autumn	summer	spring	summer
A. amnicola	73	72	69	nm
A. breweri	75	nm	72	73
A. canescens (Aus)	73	**dnp	nm	73
A. canescens (F.R.1)	72	63	nm	72
A. canescens (F.R.2)	73	nm	dnp	73
A. canescens (Rin)	76	dnp	nm	74
A. canescens (SR)	73	72	74	75
A. cinerea	72	nm	nm	nm
A. glauca	75	nm	nm	71
A. halimus	70	66	65	66
A. lentiformis	74	66	70	75
A. nummularia	68	67	71	70
A. paludosa	62	dnp	nm	nm
A. rhagadioides	70	69	nm	nm
A. semibaccata	72	nm	nm	63
A. undulata	72	nm	nm	66
Average	72	68	70	71

* not measured **did not plant

CONCLUSIONS AND RECOMMENDATIONS

During this trial some experience was gained on establishing experimental plots on different farms. Co-workers manage the experimental plots in different ways, which might have an significant influence on the results of such a trial. It is recommended that "on farm" experimental plots should be fenced off with a fence to keep out rabbits and larger rodents, as these animals are common throughout Southern Africa and problems were experienced with rabbits. This was also reported by Nord, Hartless & Nettleton (1971) in California, USA as a detrimental factor during the establishment of *A. canescens*.

It is also recommended that the species under investigation should be evaluated for other characteristics than only as fodder source. Le Houérou (1993) reported that *A. halimus* and *A. glauca* can be used for erosion control and water conservation, *A. canescens* and *A cinerea* for sand dune stabilization and almost all the species from this study for salt land reclamation and

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rehabilitation and restoration of desertified land.

The four species that are apparently the best adapted to each of the five locations, are illustrated in Table 12 . This is, however, only a suggestion. If the trial was conducted over a longer period, or planted in different seasons the results might have been different.

Table 12 The four *Atriplex* accessions that appeared to be the best adapted to each location

Pretoria	Kathu	Mier	Pofadder	Bloemfontein
A. amnicola	*A. amnicola*	*A. amnicola*	*A. canescens* (S R)	*A. canescens* (S R)
A. canescens (S R)	*A. canescens* (S R)	*A. canescens* (S R)	*A. glauca*	*A. glauca*
A. halimus	*A. lentiformis*	*A. lentiformis*	*A. lentiformis*	*A. halimus*
A. nummularia	*A. nummularia*	*A. nummularia*	*A. nummularia*	*A. nummularia*

To compare the locations intensively with one another it is recommended that the planting date and season should be synchronised. This was not the case in this study. It is also important that the edible material that is sampled from the plants should be well defined to ensure that samples from the same species do not include different proportions of woody (thick twigs) material from different locations, as in this study.

Le Houérou (1993) mentioned that a better knowledge of the ecological variability within *Atriplex* spp. is needed and from the feed value viewpoint the identification and selection of more palatable and nutritious types is still needed. It is, therefore, recommended that once a few *Atriplex* spp. are identified as adapted to a certain area or location, selection for ecotypes with certain characteristics within species should be conducted to ensure a higher production of good quality fodder in future.

From this preliminary work it is evident that there is a range of *Atriplex* material available, which is adapted to different soil and climatic conditions in the drier areas of Southern Africa. This material should now be evaluated within practical production systems as part of the "on farm" section of a research programme. Within such systems it is foreseen that plantings of *Atriplex* will reduce the pressure on rangeland and buffer the impacts of severe drought.

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CHAPTER 4

SELECTION OF *ATRIPLEX* ACCESSIONS BY GRAZING SHEEP

ABSTRACT

Grazing *Atriplex* will satisfy animal maintenance requirements, rather than production requirements. Differences in palatability as influenced by factors such as protein content, chemical composition, fibre content, odours, morphology and structure, plant growth form and growth stages have been reported. In this study the palatability of 16 different *Atriplex* accessions was investigated. The purpose was firstly, to try and observe the diet selection of sheep from 16 different *Atriplex* accessions on offer in a cafeteria system, and secondly, to determine the quality of the diet selected by sheep. Quality parameters included: *in vitro* DM digestibility (IVDMD), crude protein (CP) content, neutral detergent fibre (NDF), sodium (Na) content, percentage ash and percentage organic matter (OM). The 16 accessions which were compared were: *A. amnicola*, *A. breweri*, *A. canescens* (five accessions), *A. cinerea*, *A. glauca*, *A. halimus*, *A. lentiformis*, *A. nummularia*, *A. paludosa*, *A. rhagadioides*, *A. semibaccata* and *A. undulata*. In this trial *A. amnicola*, *A. semibaccata*, *A. rhagadioides*, *A. cinerea*, *A. halimus* and *A. canescens* (Santa Rita) were more palatable, while *A. canescens* (Rincon), *A. paludosa*, *A. canescens* (Australia) and *A. undulata* were less palatable. The palatability of other species was intermediate. The quality of the selected diets was other than expected. It had been expected that the nutritive content of the diet selected by the fistulated sheep would decrease over the grazing period, but it remained essentially the same over this period. The sheep were in fact able to select an equally nutritious diet from the relatively less palatable plants as from the relatively more palatable plants. It is suggested that this trial should be repeated with smaller numbers of accessions with similar palatability, over longer periods, to give the animals more time for selection. The amount of forage available of each species should also be quantified prior to grazing. Other livestock types and breeds might also be used to study preferences.

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INTRODUCTION

Grazing of *Atriplex* plantations, especially during dry periods, will satisfy animal maintenance requirements rather than production requirements. After good rain it is suggested that livestock be withdrawn from the veld, to give the veld an opportunity to recover and to enhance plant vigour. During this period *Atriplex nummularia* may be used for grazing (du Toit, 1992).
Le Houérou (1994) mentioned that, for South African conditions, there are other *Atriplex* species that are adapted to different conditions, which may prove better adapted than *A. nummularia* in particular circumstances, such as shallow soils, sandy soils, water-logged soils and cold zones. The species mentioned: *A. amnicola*, *A. undulata*, *A. canescens* (some types are adapted to sandy and dune habitats), *A. lentiformis* and *A. halimus* are all included in this trial.
In Chapters five and six the differences in palatability between different plants of the same species are discussed briefly. Jacobs & Smit (1977) found that there was a definite order in which individual *A. nummularia* plants, in the same camp, were grazed by sheep. This was ascribed to variation in palatability between different plants of the same species. It was also suggested that stands of *A. nummularia* should only be grazed for short periods to protect the more acceptable plants. In 1990 Pearson, Sparrow & Lange (1990) proved that prolonged grazing of an *A. vesicaria* plantation by sheep reduced the palatability of plants remaining in the population. Du Toit (1992) stressed the fact that *A. nummularia* plantations were less palatable after rainfall incidences due to accumulation of salts from the soil. He explained that palatability of *A. nummularia* stands will vary according to rain showers. Bergh (1994) tried to determine the causes of palatability differences between different plants of *A. nummularia*. Concentrations of Na, K, Ca, Mg, crude protein and neutral detergent fibre (NDF) were determined. Significant differences were found between seasons, but not between palatable and unpalatable plants. The palatable plants, however, tended to have a lower mineral content and higher crude protein and NDF content. Maywald, Mc Arthur, Jorgensen, Stevens and Walker (1998) gave experimental evidence for sex-based palatability variation in *A. canescens* with herbivores preferring to graze male shrubs during late spring. They suggested that differences in physiological vigour and/or chemistry may influence relative palatability of the sexes over time.
Ruminants discriminate between foods by their senses, sensing the consequences of food selection through feedback mechanisms, both of which are integrated within the central nervous system (Provenza, 1995). Except for the senses of the animals there are many other factors

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which may also play a role in the selection process by ruminants. Palatability, according to Heady (1964), is an attribute of the plant that the animal can recognize. These attributes includes protein content, chemical composition, fibre content, odours, morphology and structure, plant growth form and growth stages. Other factors such as soil type, temperature, rainfall, topography and mixture of species might also have an influence. The animals previous experience might also have an influence on its selection ability due to the dietary habits of the animals and also what the animal learned through previous experience. The physiological stage of the animal is equally important as well as quality and quantity of the available forage (Le Houérou, 1994). The feed selection of an animal will be influenced by a combination of all the above mentioned factors.

In this study the palatability of 12 different *Atriplex* species and five selections of one species (in total 16 different *Atriplex* accessions), was investigated. The purpose of this study, was firstly to observe the diet selection (palatable to less palatable plants) of sheep when there were 16 different *Atriplex* accessions on offer in a cafeteria system, and secondly, to determine the quality of the diet selected by sheep from the mixture of 16 *Atriplex* accessions.

MATERIAL AND METHODS

The study was conducted on the Hatfield Experimental Farm, Pretoria, South Africa. Sixteen Dohne-Merino and two Dorper wethers were used. The two Dorper sheep were oesophageally fistulated (Bredon, Torell & Marshall, 1967). Before the trial commenced the sheep grazed an established *Atriplex nummularia* plantation for two weeks. This allowed the sheep to adapt to *Atriplex* fodder before starting the trial, as was suggested by Correal (1993).

The *Atriplex* accessions, as well as the origin of each, are presented in Table 1. The experimental design and layout was a randomised block design with four randomly allocated replicates. Each replicate consisted of the 16 randomly allocated accessions. For each accession five plants were planted per replicate. The spacing was one metre within each row and three metres between the rows, which gave a density of 3333 plants per hectare (ha). This experimental area was initially used for growth and production studies.

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Table 1 Names and origin of *Atriplex* accessions evaluated

Accessions	Origin
A. amnicola Paul G. Wilson	Western Australia
A. canescens (Pursh.) (Australia)	Australia
A. canescens (Pursh.) (Rincon)	North America
A. canescens (Pursh.) (Santa Rita)	North America
A. canescens (Pursh.) (Field Reserve 1)	North America
A. canescens (Pursh.) Field Reserve 2)	North America
A. cinerea Poir.	Australia, Europe, South Africa
A. glauca L.	Mediterranean, South Africa
A. halimus L.	Asia, Mediterranean
A. lentiformis ssp. *breweri* (Wats.) Hall&Clem.	North America
A. lentiformis (Torr.) Wats. ssp. *lentiformis*	North America
A. nummularia L.	Australia
A. paludosa R. Br.	Australia
A. rhagadioides F. Muell.	Australia
A. semibaccata R. Br.	The Americas, Europe, Australia & South Africa
A. undulata D. Dietr.	South America

For the grazing trial the four replicates were fenced separately to give four paddocks, each measuring 20 metres by 11 metres. Within each of the four camps all the plants, except two plants of each accession, had been clipped to ten centimetres. The two plants of each accession remaining unclipped in each camp were treated uniformly in the months prior to this trial and were left to be grazed during this trial. The 32 plants (two of each of the 16 *Atriplex* accessions) were now made available in a cafeteria system for the sheep to choose and graze the more palatable plants. Each paddock was supplied with two watering points to make sure the animals did not concentrate on the plants nearest to one watering point. Before grazing commenced the height and width of all plants was measured. After grazing these measurements were repeated. Photographs of all plants were taken prior to, during and after grazing. These photographs will be used to illustrate the differences in defoliation of the different accessions.

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Four Dohne-Merino wethers were placed in each of the four paddocks (density of 180 wethers per ha) and allowed to graze from eight o clock in the morning until three o clock in the afternoon. The remainder of each day and night was spent in a shelter. To determine which plants were more palatable the technique of direct observation, as described by Lange & Willcoks (1978) and Holechek, Vavra & Pieper (1982), was used. The grazing sheep, were observed continuously from an elevated position and a score were kept of each plant that was grazed at a certain time in each replicate. Measurements were taken over a nine day period. The observations were made throughout the grazing period and scores were taken as one mark for each animal that visited a certain *Atriplex* accession. On the scoring sheet the four paddocks and the names of the 16 *Atriplex* accessions were listed. The scores were ticked against the *Atriplex* accession grazed.

The two fistulated Dorper sheep were rotated daily from camp to camp with two cycles being completed in eight days. These sheep were kept in a separate camp without feed for three hours, before grazing. The plugs (from the oesophageal fistulas) were then removed, the collection bags fitted and the sheep placed in the camp and allowed to graze for 30 minutes. During the 30 minutes these sheep were observed and the plants they visited were noted. After 30 minutes of grazing the sheep were returned to the separate camp again, the collection bags were removed and a plus minus 500 g. sample was taken from each collection bag. The animals were then allowed to graze, in the same camp from which the samples had been taken, for the remainder of the grazing period. Fistula samples were squeezed in coarse cotton cloth to eliminate most of the saliva. The samples were then frozen. The frozen samples were freeze dried and milled. The following chemical analyses were conducted to determine nutritive quality of each sample: *in vitro* dry matter digestibility (IVDMD), using the Tilley and Terrey technique, as adapted by van der Merwe (van der Merwe & Smith, 1991); neutral detergent fibre (NDF), using the Dosi Fibre system with the Robbertson and Van Soest method (Robertson & Van Soest, 1981); sodium (Na) using a wet calcination and sulphur technique; and crude protein (CP)/N-content, using the Kjeldahl technique (AOAC, 1984). Other measurements included dry matter (DM), organic matter (OM) and ash contents which were determined by oven drying. Dry matter was determined by weighing two grams of the sample, drying it at 100°C for 24 hours or until constant weight. Ash was determined by incinerating weighed samples in an oven at 600°C for 4 hours. Organic matter was determined as the difference between DM and ash.

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RESULTS AND DISCUSSION

Palatability - using direct observation

Throughout the nine day period the animals were directly observed, to identify the palatable or preferred plants. A percentage was calculated for the number of times which each plant was visited each day. If the total visits on one day to all the plants was 100% then the percentage worked out for a specific plant was the number of visits that plant received in relation to the total amount of visits. This data is presented in Table 2.

Table 2 The six most frequently grazed accessions on each of the nine days. Percentage was the contribution of each of the 16 accessions to the total of 100%

Grazing day and 6 mostly grazed spp. on each day	% of total for each day	Grazing day and 6 mostly grazed spp. on each day	% of total for each day	Grazing day and 6 mostly grazed spp. on each day	% of total for each day
1		2		3	
A. amnicola	26	*A. amnicola*	21	*A. semibaccata*	23
A. semibaccata	17	*A. semibaccata*	21	*A. canescens* (S.R.)	11
A. rhagadioides	14	*A. canescens* (S.R.)	11	*A. halimus*	11
A. cinerea	8	*A. glauca*	10	*A. amnicola*	10
A. glauca	8	*A. rhagadioides*	9	*A. undulata*	10
A. undulata	5	*A. halimus*	6	*A. glauca*	7
4		5		6	
A. amnicola	19	*A. amnicola*	14	*A. lentiformis*	13
A. halimus	15	*A. halimus*	14	*A. cinerea*	11
A. semibaccata	15	*A. cinerea*	9	*A. amnicola*	9
A. canescens (S.R.)	8	*A. semibaccata*	9	*A. canescens* (F.R.1)	9
A. cinerea	8	*A. canescens* (S.R.)	8	*A. canescens* (S.R.)	8
A. paludosa	6	*A. nummularia*	8	*A. rhagadioides*	8
7		8		9	
A. nummularia	12	*A. nummularia*	23	*A. nummularia*	15
A. halimus	11	*A. canescens* (S.R.)	13	*A. halimus*	15
A. breweri	9	*A. canescens* (F.R.2)	10	*A. breweri*	9
A. semibaccata	8	*A. halimus*	9	*A. canescens* (F.R.1)	9
A. canescens (F.R.1)	8	*A. paludosa*	7	*A. canescens* (S.R.)	9
A. canescens (F.R.2)	8	*A. lentiformis*	6	*A. canescens* (F.R.2)	8

The hypothesis was that the animals would first graze the most palatable plants and as soon as the edible parts of these plants became depleted, the animals would move to the next group of less palatable accessions. The period over which this trial was conducted was short enough to

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prevent the animals from returning to new regrowth on the already grazed plants. From the data in Table 2 it is clear that the animals preferred *A. amnicola* during the first five days, with visits ranging from 10% to 26%. Other preferred accessions during the first five days were *A. semibaccata*, *A. canescens* (S.R.) and *A. halimus*. *Atriplex halimus* and *A. canescens* (S.R.) were, with the exception of days one and six and days one and seven respectively, always among the six mostly preferred accessions. This might be because of the size of these two accessions which were much taller and wider than most of the other plants and therefore produced more edible material than most of the other accessions. During the last three days *A. nummularia* tended to be the most palatable plant with visits ranging from 12% to 23%. Other important accessions during the last three days were *A. breweri* and *A. halimus*. It is difficult to isolate one accession as the most palatable as there were many errors that could be made by the observer during the collection of the data and there were 16 accessions on offer. It would, therefore, be safer to identify a group of plants which were more palatable and a group of plants which were less palatable.

Figure 1 shows the average percentage of visits over the nine day period. The six most visited, and therefore, more palatable plants were: *A. amnicola* (12.44%), *A. semibaccata* (11.48%), *A. halimus* (10.46%), *A. canescens* (S.R.) (8.65%), *A. nummularia* (7.61%) and *A. cinerea* (7.14%). The five plants that were visited the least, and therefore less palatable, were: *A. canescens* Rincon (1.96%), *A. canescens* Austr. (2.49%), *A. paludosa* (3.18%), *A. undulata* (3.51%) and *A. canescens* (F.R.2) (4.21%). The percentage of visits to other plants were intermediate. These data are more or less in line with that of Jacobs & Smit (1977) who reported that sheep will first graze *A. nummularia* and then *A. canescens* and then *A. breweri* and *A. lentiformis*. In this trial five different accessions of *A. canescens* were tested and a high variability in their palatability was experienced. According to Correal (1993), five *Atriplex* species were ranked as follows according to its palatability: *A. repanda* (most palatable), *A. nummularia*, *A. amnicola*, *A. halimus*, *A. canescens* and *A. cinerea* (least palatable). This ranking differ from the findings of this trial, but this might be because Correal (1993) also took persistence (recovery) after grazing into consideration when ranking the plants.

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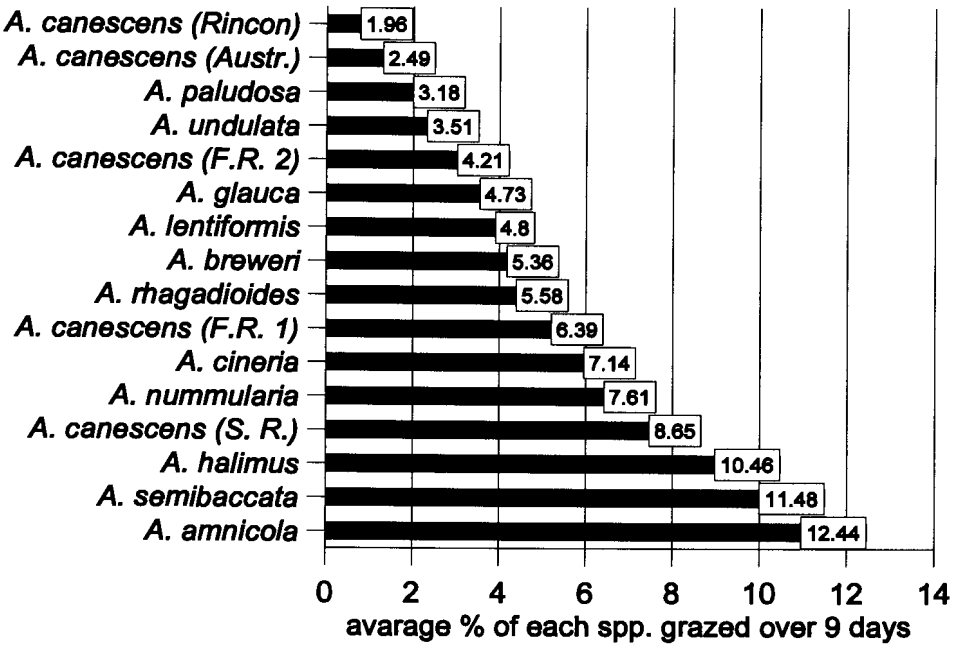

Figure 1 The average percentage of visits to 16 accessions for the entire duration of the trial

The overall conclusion from the data in Table 2 and Figure 1 is that *A. amnicola*, *A. semibaccata*, *A. halimus*, *A. canescens* (S.R.) and *A. nummularia* were the more palatable accessions while *A. canescens* (Rincon), *A. canescens* (Austr.), *A. paludosa* and *A. undulata* are the least palatable accessions.

To quantify the rate at which each accession was utilized over the nine day period, each of the plants was measured before grazing and again after the trial was finished. Measurements included plant height and plant width (diameter). Plant volume was calculated using the formula for the determination of the volume of a cylinder ($\pi r^2 h$). Where r is half of the plant diameter and h is the plant height. These results are presented in Table 3.

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Table 3 Average plant height, diameter and volume, before and after grazing

Accessions	Plant measurements						Reduction in height, diameter and volume during grazing period			Reduction in height, diameter and volume during grazing period		
	Before grazing			After grazing								
	Height	Diameter	Volume	Height	Diameter	Volume	Height	Diameter	Volume	Height	Diameter	Volume
	(m)		(m^3)	(m)		(m^3)	(m)		(m^3)	%		
A. amnicola	0.90	1.84	2.40	0.64	1.53	1.17	0.26	0.31	1.22	29	17	51
A. breweri	0.79	1.60	1.58	0.64	1.36	0.93	0.15	0.24	0.65	19	15	41
A. canescens (Austr.)	1.09	1.46	1.81	0.89	1.33	1.24	0.20	0.13	0.57	18	9	32
A. canescens (F.R. 1)	0.81	1.41	1.26	0.66	1.15	0.69	0.15	0.26	0.57	19	14	45
A. canescens (F.R. 2)	0.81	1.23	0.97	0.59	1.00	0.46	0.22	0.23	0.51	27	19	52
A. canescens (Rincon)	0.59	0.83	0.32	0.48	0.82	0.26	0.11	0.01	0.06	19	1	20
A. canescens (S. R.)	1.24	1.55	2.34	1.02	1.25	1.24	0.22	0.30	1.09	18	19	47
A. cinerea	0.48	1.21	0.55	0.33	0.90	0.21	0.15	0.31	0.33	31	26	61
A. glauca	0.67	1.34	0.95	0.49	1.07	0.45	0.18	0.27	0.50	27	20	53
A. halimus	1.25	1.73	2.92	1.17	1.58	2.28	0.08	0.15	0.64	6	9	22
A. lentiformis	0.88	1.23	1.03	0.71	1.06	0.62	0.17	0.17	0.41	19	14	40
A. nummularia	1.29	1.68	2.87	1.16	1.56	2.21	0.13	0.13	0.66	10	8	23
A. paludosa	0.44	0.69	0.17	0.29	0.62	0.09	0.15	0.07	0.08	34	10	48
A. rhagadioides	0.56	1.24	0.67	0.21	0.63	0.07	0.35	0.61	0.61	63	49	90
A. semibaccata	0.32	2.27	1.29	0.13	1.65	0.27	0.19	0.62	1.01	59	27	79
A. undulata	0.61	0.95	0.43	0.43	0.80	0.22	0.18	0.15	0.21	30	16	50

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There were big differences in the volumes of the 16 accessions before the grazing started, as can be seen in Table 3. This is due to their different growth forms. The implication is that there were different amounts (quantities) of the different accessions available for grazing. According to Le Houérou (1994), the quantity and quality of the available forage will influence the diet selection of animals. The structure and growth form together with the quantity and quality of each accessions might thus have had an influence on the acceptability of each accession. In Table 3 it can be seen that the taller plants like *A. halimus* and *A. nummularia* had a small reduction in plant height of 0.08 m and 0.13 m respectively, while shorter plants, such as *A. amnicola* and *A. rhagadioides*, had relatively much larger reductions in height of 0.26 m and 0.35 m respectively. From this it may be concluded that sheep will prefer plants which are growing within easy reach, to plants that are growing outside the animals average grazing height. What is, however, interesting is that *A. canescens* (S.R.), which is a tall plant, showed a height reduction of 0.22 m, which was relatively greater than that of the other tall plants mentioned. This might be an expression that *A. canescens* (S.R.) was more palatable to sheep than *A. nummularia* and *A. halimus*.

With respect to the plant volumes, a good example of interacting factors is *A. paludosa*. The volume before grazing was 0.17 m^3 and after grazing 0.09 m^3. The sheep spent only 3 % of their total grazing time on this accession (Figure1). Due to its small volume, all the edible material would have been utilized over a short period, whereas if there had been more plants available, to offer the same amount of edible material as some of the larger accessions, the sheep might have spent more time grazing this accession. To try and refine the quantification of the utilization of each accession the average reduction in volume of each accession was determined as a percentage of the plant volume before grazing. The data is presented in Figure 2. The three accessions with the highest reduction in volume were *A. rhagadioides* (90%), *A. semibaccata* (79%) and *A. cinerea* (61%). These three accessions together with *A. amnicola* (51%) were more herbaceous and less woody than the other accessions. This might explain the high reduction in volume of these plants, as more of the twigs were also browsed.

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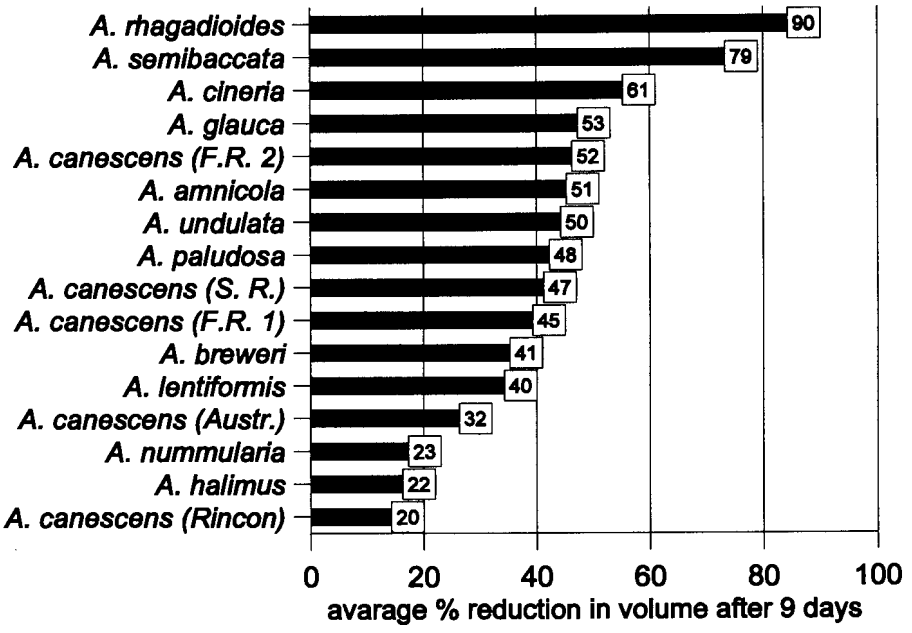

Figure 2 Average percentage reduction in plant volume of the 16 accessions after nine days of grazing

The three accessions with the lowest reduction in volume were *A. canescens* Rincon (20%), *A. halimus* (22%) and *A. nummularia* (23%). These three accessions were more woody than the other accessions, and only the thinnest twigs were browsed. This method of determining the plant volume does, however, have its limitations. The plant height and width is measured by measuring from and to the stem tips, which means that all the leaves that were grazed from the thicker stems and all the young twigs in the middle of the plant that were browsed were not measured or quantified. To overcome this problem a method as described by Pearson *et al* (1990) where terminal shoots were tagged should be considered. After the grazing period, the loss of tagged terminal shoots could then be determined to quantify the utilization of each plant.

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Quality of selected diet - using oesophageal fistulated sheep

The two oesophageal fistulated Dorper sheep started on day one in paddock two, day two they were in paddock four, day three in paddock three and day four in paddock one. On day five no samples were taken as it was weekend and logistical problems were experienced. On day six the oesophageally fistulated sheep started again in paddock two, day eight in paddock four and day nine in paddock three. A sample was taken on day seven from paddock one, but it was mislaid during transportation from one refrigerator to another. The edible plant material of all the accessions in paddock one was virtually completely utilized and the sheep had to be withdrawn from that paddock on day seven and this was why the fistulated sheep visited this paddock out of sequence. These samples were analysed to determine the nutritive value of the diets which the sheep selected on each day. The results are presented in Table 4. Analysis of variance was conducted and the means in the same column with different alphabetical letters differed significantly ($p<0.05$). The animals were observed for 30 minutes while grazing and the six mostly visited accessions are also named in the table, together with the percentage each contributed to the total number of visits. Examining the accessions most grazed by the fistulated sheep (Table 4), it is difficult to determine a pattern of preference for the different accessions over time. During this trial these sheep were very hungry when they started grazing, because they had been pre-starved, and when entering a paddock for the first time they would run to the nearest plant and start to graze it immediately. After a while they would start to move around in the paddock, usually to plants where other sheep were grazing. They would stop at one plant and graze it for a reasonable while. The plants grazed for the longest periods were *A. semibaccata* on days one, two and three, *A. breweri* and *A. lentiformis* on day four, *A. canescens* (F.R.1) on day six, *A. canescens* (F.R.2) and *A. canescens* (Australia) on day eight and *A. halimus* on day nine. The more important information is the quality of the diet selected by sheep. Over time, as the relatively more palatable plants became depleted, the sheep were forced the graze the relatively less palatable plants. The percentage OM, Ash and Na of the selected material remained more or less the same throughout the period. The percentage ash in the selected diet was on average about 19 %, which was lower than the 26 % determined for seven *Atriplex* accessions by Colomer & Passera (1990). Although other factors such as soil chemical composition might also have an influence on the ash content, these data indicate that sheep might prefer to select a diet with a lower ash content. The CP content varied significantly over time.

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Table 4 Results of the analysis for the nutritive value of oesophageal samples taken from sheep grazing 16 different *Atriplex* accessions

Grazing day and species grazed on each day	% grazed	OM %(DM)	Ash %(DM)	Na %(DM)	CP %(DM)	NDF %(DM)	IVDMD %(DM)	Paddock nr.
1 (a)		83 ^{d,e,g}	17 ^{d,e,g}	3	18 ^{b,c,d,f,g}	37± 5	71 ± 5 ^{f}	**2**
A. semibaccata	31							
A. breweri	13							
A. cinerea	13							
A. glauca	13							
A. nummularia	13							
A. amnicola	6							
2 (b)		83 ^{d,e,g}	17 ^{d,e,g}	3	13 ^{a}	40 ± 6	67 ± 3	**4**
A. cinerea	27							
A. semibaccata	27							
A. breweri	13							
A. nummularia	13							
A. canescens (S.R.)	7							
A. rhagadioides	7							
3 (c)		83 ^{d,e,g}	17 ^{d,e,g}	3	12 ^{d,e}	44 ± 0 ^{d,e}	64 ± 5 ^{e}	**3**
A. semibaccata	29							
A. halimus	24							
A. amnicola	12							
A. cinerea	12							
A. nummularia	12							
A. rhagadioides	12							
4 (d)		80 ^{a,b,c}	20 ^{a,b,c}	3	14 ^{a}	34 ± 0 ^{c}	72 ± 0 ^{f}	**1**
A. breweri	16							
A. lentiformis	16							
A. canescens (F.R.1)	13							
A. nummularia	13							
A. canescens (S.R.)	9							
A. halimus	9							
6 (e)		80 ^{a,b,c}	20 ^{a,b,c}	3	17 ^{c,g}	32 ± 2 ^{c,f}	74 ± 2 ^{c,f}	**2**
A. canescens (F.R.1)	23							
A. halimus	18							
A. nummularia	14							
A. amnicola	9							
A. glauca	9							
A. lentiformis	9							
8 (f)		81	19	2	13 ^{a}	42 ± 5 ^{e}	60 ± 4 ^{a,d,e}	**4**
A. canescens (F.R.2)	22							
A. canescens (Austr.)	22							
A. nummularia	17							
A. breweri	17							
A. amnicola	6							
A. paludosa	6							
9 (g)		79 ^{a,b,c}	21 ^{a,b,c}	3	11 ^{a,e}	39 ± 2	67± 2	**3**
A. halimus	35							
A. canescens (F.R.2)	25							
A. canescens (Rincon)	10							
A. paludosa	10							
A. amnicola	5							
A. nummularia	5							

Superscript a-g: Means in the same column, differ significantly ($P<0.05$) from means of grazing days marked with the same letter

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What is, however, interesting is the fact that the variation was between paddocks and not actually over time as was expected. The CP content for paddock two was 18% on day one and again 17% on day six. For paddock four the CP content was 13% on day two and 14% on day four. Paddock three had a CP content of 12% on day three and of 11% on day nine. This might be explained as a variation in the amount of nutritious, edible plant material between the different paddocks. Even though the palatable plants were depleted to some extent after the first visit the sheep were still able to select a diet with the same CP content during the second visit. The accessions most frequently grazed in paddock two differed, with the exception of *A. amnicola*, *A. glauca* and *A. nummularia*, between days one and six and yet the CP content remained essentially the same.

The percentage NDF also exhibited significant variation between paddocks but not over time. Over time it was postulated that the percentage NDF would have increased as the herbaceous material became depleted and the animals were forced to graze more fibrous woody material. Paddock two had low NDF values of 37% and 32% for days one and six respectively which, together with high CP and *in vitro* dry matter digestibility (IVDMD) values, indicated that this paddock had more nutritious material on offer than the other paddocks. The IVDMD also varied significantly between paddocks but not over time. Paddock four had a lower IVDMD of 60% on day eight while it was 67% on day two, but this decrease was not significant.

These data were other than what had been expected. It had been expected that the nutritive content of the diet selected by the fistulated sheep would decrease over time, but it remained essentially the same. The sheep were able to select an equally nutritious diet from the relatively less palatable plants as from the relatively more palatable plants. The data also revealed the variation in total nutritive composition that existed between paddocks.

A limitation of this trial was the amount of edible material of each accession on offer in each of the four paddocks. According to Provenza (1995) goats first introduced to blackbrush (*Coleogyne ramosissima*) dominated rangelands, sampled all foods in the area during the first few hours, including potentially toxic plants as well as non-toxic plants. They also sampled both current season growth and old growth of blackbrush. The goats at first ate small amounts of each type of food on offer, and during the next few days they limited intake of the potentially toxic foods and ingested materials composed primarily by blackbrush old growth, containing less tannins than current seasons growth of blackbrush. The plant material in this trial was, however,

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not sufficient for the animals to make a distinguished selection between the different accessions, as the edible material in paddock number one was already finished on day seven. In future, fewer accessions should be used to limit observer errors, and more edible material of each accession should be on offer to give the animals more time to select between the accessions.

According to Holechek *et al* (1982) difficulty in species identification and quantification of how much of a plant was consumed are important problems associated with the direct observation technique that was used for this trial. In this case, however, the species identification was not a problem as only 32 plants (two of each accession) were present in each paddock and the plants were numbered. The quantification was, however, neglected. Holechek *et al* (1982) stressed that quantitative information from direct observation has been obtained from the bite-count and feeding minutes. Feeding minutes is where the time spent grazing each species is quantified and assumed to be proportional to the importance of the species in the diet. In our trial the time spent at each species was indirectly represented in the data as the observations were made rotationally. Observations were made in paddocks one, two, three and four sequentially and were started again at paddock one. If an animal was still grazing at the same plant as when the first observations were made, the same plant would be scored again, and the higher the score for a plant, the more time was spent at that specific plant. Another method for direct observation and quantification of the amount of feed taken in by the animal is described by Heady (1964), who used penned animals which were given a choice of a known amount of two or three foods. Pearson *et al* (1990) described a method where terminal shoots of *A. vesicaria* were tagged. They compared different populations of *A. vesicaria* for palatability. Relative palatability of the populations was assessed by their rates of decline in bush biomass, as indexed by loss of tagged terminal shoots. Methods, other than direct observation, that have been used to determine the botanical composition of herbivore diets are oesophageal fistulas, rumen fistulas (stomach content analysis) and faecal analysis (McInnis, Vavra & Krueger, 1983; Mohammad, Pieper, Wallace, Holechek & Murray, 1995). Of all these techniques the oesophageal fistula method is reputed to be the most accurate. The oesophageal fistula technique is usually used to determine the botanical composition of range herbivore diets. The samples from fistulated animals are analysed microscopically and this is called microhistological analysis (Alipayo, Valdez, Holecheck & Cardenas, 1992). In this study, however, this technique was only used to determine the quality of the selected diet and not the botanical composition of the selected diet.

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Photographic evaluation

Examples of photographs taken before and after the grazing trial are presented in Figures three to six. These photographs give an indication of the utilization of certain of the accessions. For the photographs a grid was placed behind the plants to give a comparative scale. The grid that was used was 1.2 metres by 1.5 metres. Each of the blocks on the grid had a size of 10 cm. by 10 cm. In Figure 3 the two mostly grazed accessions, *A. amnicola* and *A. semibaccata* are shown. It can be seen that *A. semibaccata* were totally defoliated but most of the stems were not grazed. On the other hand *A. amnicola*, which is one of the more herbaceous accessions, was not only totally defoliated, but a large portion of the stems were also removed. This illustrates the earlier point that plant measurements (height and diameter) after grazing are often not fully representative of the situation, as the measurements are taken from and to shoot tips and did not include all the leaves, which were stripped from the stems. The photographs, therefore, give a better indication of the extent to which each accession was grazed, than the plant measurements alone.

Figure 4 shows *A. canescens* (Rincon) and *A. paludosa*, which were grazed the least and were, therefore, classed less palatable plants. From the photographs it can be seen that *A. canescens* (Rincon) was only lightly grazed with most of the leaves still on the stems, while *A. paludosa* was almost untouched. There were some *A. paludosa* plants that were grazed more than that in Figure 4, but those plants were in paddock one where virtually all the edible material was depleted after seven days and the animals were forced to graze all the plants in the paddock as it was the only feed still available.

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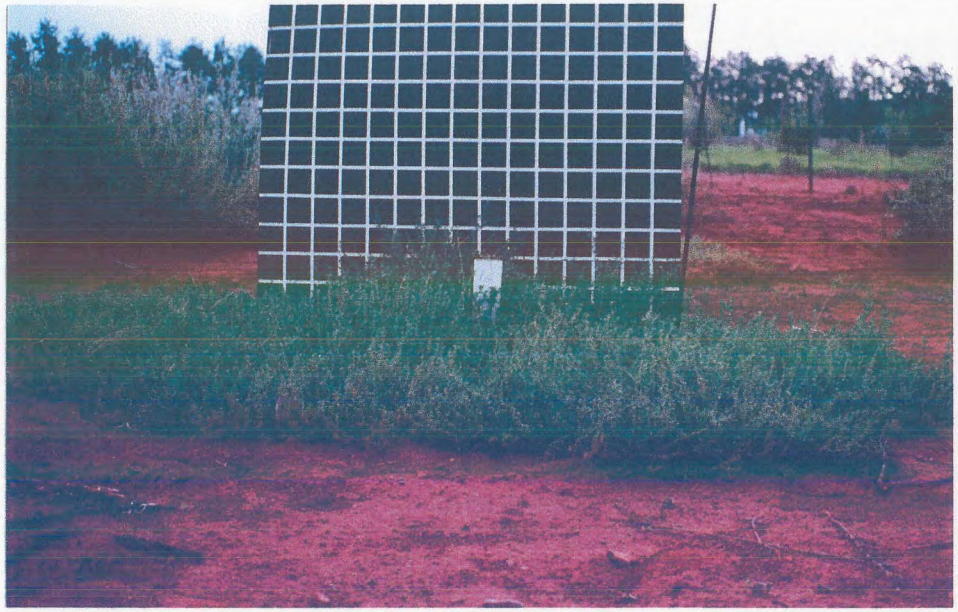

A. semibaccata (before grazing)

A. semibaccata (after grazing)

A. amnicola (before grazing)

A. amnicola (after grazing)

Figure 3 Photographs of *A. semibaccata* re and after grazing. For *A. amnicola* two adjacent plants were photographed

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A. canescens Rincon (before grazing)

A. canescens Rincon (after grazing)

A. paludosa (before grazing)

A. paludosa (after grazing)

Figure 4 Photographs of *A. canescens* (Rincon) and *A. paludosa* before and after grazing

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In Figure 5 the photographs of *A. rhagadioides* and *A. canescens* (Santa Rita) can be seen before and after grazing. *Atriplex rhagadioides* is the accession with the highest reduction in plant volume of 90% (Figure 2). On the photograph it can be seen that *A. rhagadioides* was completely defoliated and that a large proportion of the stems were also grazed. The *A. canescens* accession which was well utilized was Santa Rita. It showed a reduction in volume of only 47% (Figure 2), but on the photograph in Figure 5 it can be seen that virtually all the leaves were stripped from the stems to a height of more or less 1.2 metres. *Atriplex canescens* is generally more woody than most of the other *Atriplex* species and only the thinner stems were, therefore, grazed.

In Figure 6 photographs of *A. halimus* and *A. nummularia*, before and after grazing, are presented. *Atriplex halimus* was the only species that was, with the exception of two days, amongst the six most grazed accessions throughout the trial. Although it exhibited a reduction in volume of only 22% (Figure 2), it was the third most grazed (10.46%) of all the accessions (Figure 1). In Figure 6 it can be seen that *A. halimus* was grazed very well and that almost all the leaves were utilized. The stems, however, are woody and only a small proportion were grazed. Even though it had a relatively small reduction in volume, *A. halimus* was very well utilized as one of the more palatable plants. *Atriplex nummularia*, which is well known and widely used in South Africa, was one of the less palatable plants and in Figure 6 it can be seen that *A. nummularia* was lightly grazed, but not to the same extent as most of the other accessions.

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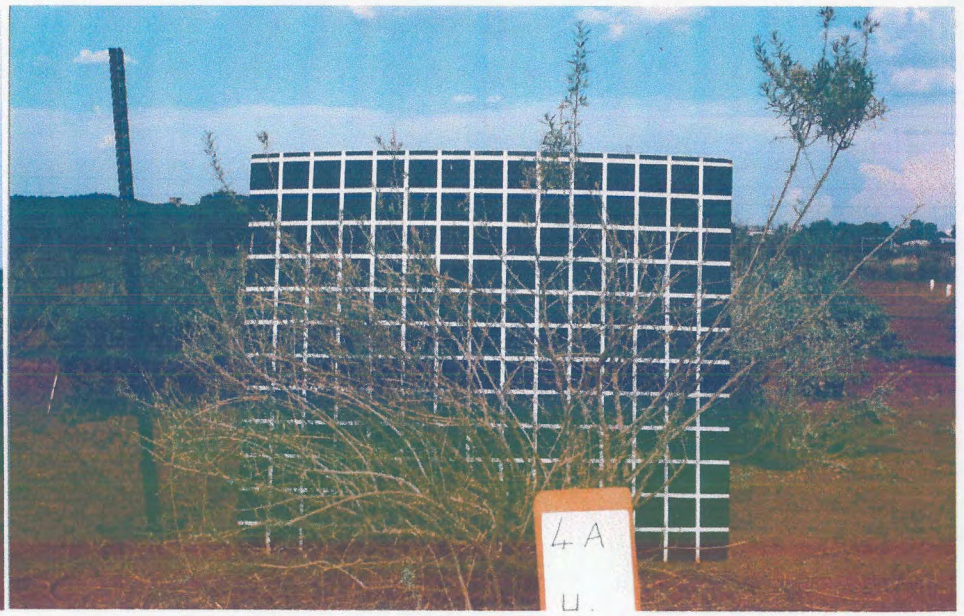
A. rhagadioides (before grazing)

A. rhagadioides (after grazing)

A. canescens Santa Rita (before grazing)



A. canescens Santa Rita (after grazing)

Figure 5 Photographs of *A. rhagadi* a Rita) before and after grazing

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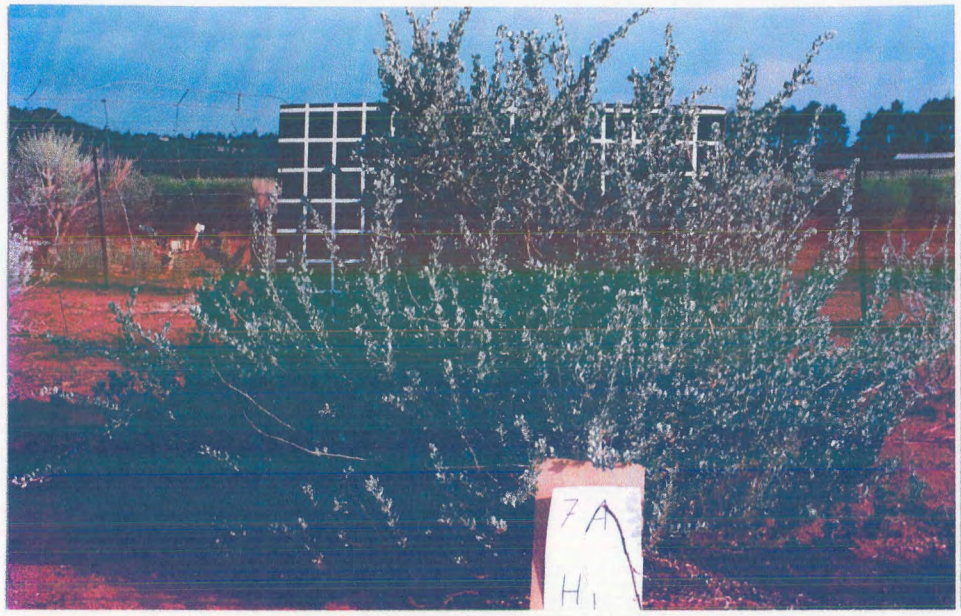
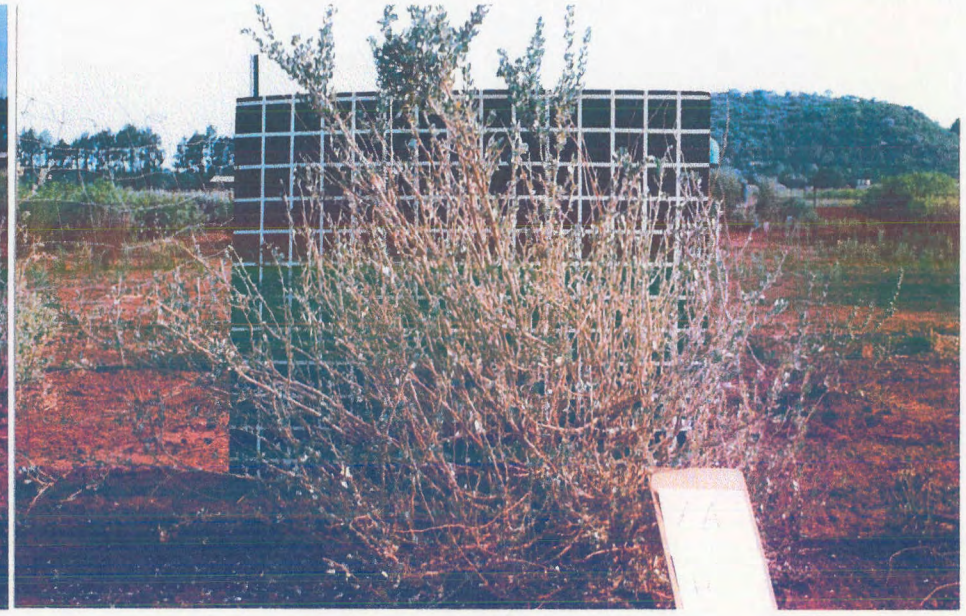
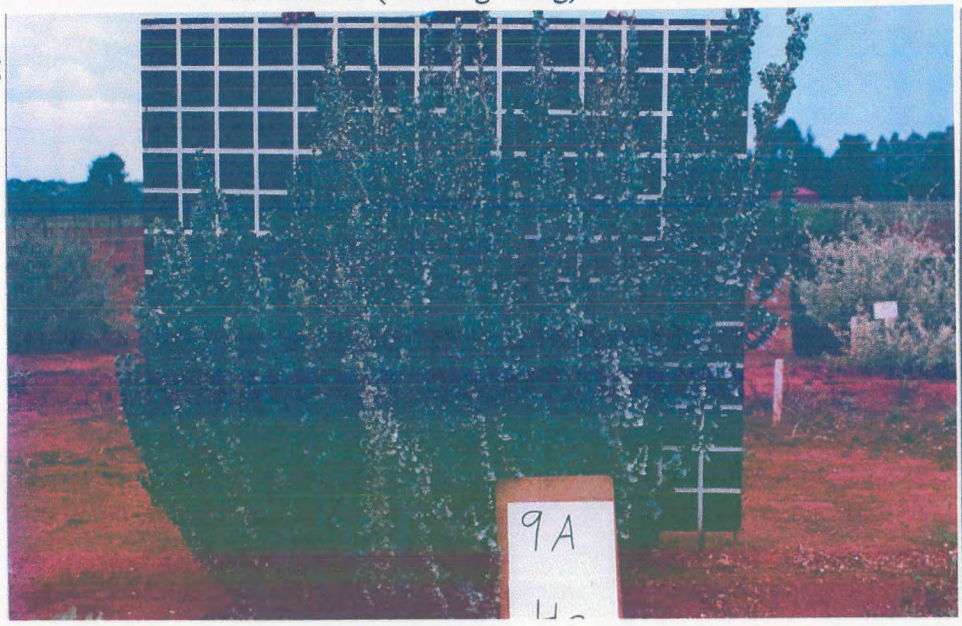


A. halimus (before grazing)



A. halimus (after grazing)



A. nummularia (before grazing)

A. nummularia (after grazing)

Figure 6 Photographs of *A. halimus* a l after grazing

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CONCLUSIONS AND RECOMMENDATIONS

Much was learnt about palatability studies in the cafeteria system used in this trial. Ruminants acquire strong preferences for foods early in life, and prefer those foods as adults, whereas they are often reluctant to eat those they have not experienced (Provenza, 1995). Even though the sheep were exposed to *Atriplex* fodder for a two week period prior to the trial, sheep that started grazing *Atriplex* species at a young age might have had other preferences.

Du Toit, Blom & Immelman (1995) found that the diet selection of Afrino, Dorper and Merino Sheep and Angora goats differed only 4% to 5% during the growing season while they differed 14% to 21% during the dormant season. This stresses the influence of growth season on the diet selection of different small stock species. According to Ibrahim (1981), perhaps the most important single factor affecting palatability of a given species, or group of species, is the seasonal changes in plant growth. Palatability variation of *Atriplex* species over seasons was mentioned by du Toit (1992) and Maywald *et al* (1998). It is, therefore, recommended that a study with the most promising *Atriplex* accessions from this trial should be conducted, where the plants are grazed in the four different seasons to evaluate the effect of season on palatability.

An example of different diet selections by different small stock species is that of Dorper and Merino sheep and Angora goats, which selected very similar diets, while the diet selected by Boer goats differed by approximately 30% from those of the other animals (du Toit & Blom, 1995). According to Malechek & Narjisse (1990) the diet selection of goats is usually more diverse and exhibits seasonal shifts among grasses, forbs, and browse, favouring the most nutritious plant species and parts at different times. In contrast sheep will maintain a relatively stable dietary botanical composition and eventually compensate for low nutritive value by increasing their ingestion of fibrous mature forages. Zeeman, Marais & Coetsee (1983), however, indicated that the digestibility of organic matter selected by Dorper and Merino sheep was higher than the selection made by Boer goats with the selection by cattle having the lowest digestibility. Merino and Dorper sheep also selected diets with a higher nitrogen content than those selected by goats and cattle (Zeeman, Marais & Coetsee, 1984). It is, therefore, suggested that this study should be repeated, using different sheep and goat breeds.

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Results of a palatability study should also be expressed in a way which illustrates relative use in relation to relative availability (Heady, 1964). In this trial each of the *Atriplex* plants contributed different amounts to the total dry matter yield of each paddock. A correlation was made between the total average percentage of edible material, of each accession, available in each paddock (earlier in the season), and the average percentage grazing time spent at each accession. A r^2 value of 44% were obtained. This correlation was relatively low. If much less than 16 accession are compared in future, for example only four, better data sets can be obtained for making such correlations, as there will be fewer different accessions contributing to the total DM production.

It is strongly recommended that smaller numbers of accessions should be used in future studies. It is difficult to compare 16 accessions simultaneously, as there are too many factors influencing the diet selection of the animal. It is suggested that the four most palatable plants from this trial should be planted in larger numbers, to conduct another grazing trial comparing the palatability of these plants.

Correal (1993) studied the recovery, after grazing, of several *Atriplex* species and used this, together with the palatability of the plants, to rank the plants in terms of acceptability as fodder shrubs. This should also be done in future trials to determine whether the palatable accessions will persist under grazing. It is important to be aware of all the factors that might influence such cafeteria trials. Such trials need careful planning and the execution of such trials should be done with utmost care as there are many factors which might play a role.

In this trial *A. amnicola*, *A. semibaccata*, *A. rhagadioides*, *A. cinerea*, *A. halimus* and *A. canescens* (Santa Rita) were more palatable, while *A. canescens* (Rincon), *A. paludosa*, *A. canescens* (Australia) and *A. undulata* were less palatable. The palatability of the other species, *A. canescens* (F.R.1), *A. canescens* (F.R.2), *A. breweri*, *A. lentiformis*, *A. glauca* and *A. nummularia*, were intermediate. It is suggested that this trial should be repeated with fewer accessions, which have been identified as accessions with similar palatability. This will refine the ranking of accessions from the most palatable to the least palatable. More plants of each accession should also be used to allow the trial to proceed for a longer period, which will give the animals more time for selection. The amount of forage available of each species should also be quantified prior to the trial. One of the methods described in the introduction might be used to quantify the amount of edible material of each accession. Other livestock types and breeds might also be used to study preferences.

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CHAPTER5

IN VITRO PROPAGATION OF ELITE *ATRIPLEX* SHRUBS

ABSTRACT

Differences in palatability of *Atriplex nummularia* brought about the need for producing palatable plants. As this species is open pollinated it was decided to use a vegetative method of propagation. *Atriplex nummularia* and *A. canescens* shrubs were selected and used to develop a procedure to propagate them *in vitro*. This study was started in Israel and continued and completed in South Africa. The MS basal medium with the addition of different concentrations BA and NAA was used throughout the study. The best disinfestation method was to wash cuttings in 0.3% liquid detergent, rinse in running tap water, wash in 0.5% benlate (five minutes), wash in 0.7% sodium hypochlorite (six minutes), rinse four times in sterile distilled water (10 minutes). During culture growing the following measurements were taken: plant height; numbers of shoots, leaves and buds; and percentage cultures with callus and with roots. For *A. canescens* the best medium for shoot multiplication and elongation was MS basal medium with addition of 0.20 $mg.l^{-1}$ BA and 1.0 $mg.l^{-1}$ NAA, while a medium with 0.05 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA was good for elongation and rooting. For *A. nummularia* the best medium for multiplication was MS basal medium with 0.20 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA, while the MS basal medium without any plant growth regulators were the best for elongation and rooting. Average survival rate after acclimatization was 89%. Growth medium was washed from plant roots, and plants were planted in pots containing a mixture (1:1) of sand and peat. Plants were treated with a mist spray under a shade cloth. The intervals of the mist spray were gradually increased while the shade cloth was gradually removed. It is recommended that the procedure for *A. canescens* should be studied further, especially for a rooting medium. The procedure for *A. nummularia* just needs refinements refinements.

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INTRODUCTION

Atriplex nummularia (Oldman Saltbush) is a well-known fodder shrub in the drier parts of South Africa. On the Hatfield Experimental Farm differences in the palatability of individual plants were recorded. During browsing trials it was observed that sheep selected certain shrubs while other shrubs were not browsed at all. This phenomenon was also observed by other authors for other *Atriplex* species. Pearson, Sparrow & Lange (1990) examined the variability in palatability of populations of *Atriplex vesicaria*. It was also found that sheep preferred male plants of *Atriplex canescens* to female plants during certain times of the year (Maywald, Mc Arthur, Jorgensen, Stevens & Walker, 1998). The sheep first browsed the more palatable plants and also to a greater extent than the less palatable plants. This may cause a decrease in palatable plants and an increase in less palatable plants over time and the uniformity, concerning palatability, of the *Atriplex* stand would deteriorate. To overcome this problem it was decided that the more palatable plants should be selected for and multiplied. A method for vegetative propagation was selected as the necessary approach to multiply selections, because *Atriplex* species are dioecious and open pollinated, and seed can not, therefore, be used.

Micropropagation (plant tissue culture) was chosen. This involves the *in vitro* culture of existing meristems resulting in shoot multiplication. Roots can be induced from these shoots by either transferring to an auxin- containing medium or chemical treatment prior to the establishment in a greenhouse (Bhojwani, 1990). Perennial crops which exhibit continuous growth throughout the year, as is the case with *Atriplex* species, are easier to stabilise in cultures while those which show highly seasonal growth are more difficult to culture (Bhojwani,1990). This favours the propagation of *Atriplex* species using tissue culture.

Earlier plant tissue culture technology was aimed primarily at mass propagation but this technology is now also being used in genetic improvement programmes (Wochok & Sluis, 1980). Ferreira (1995) stressed the worlds continuously growing demand for more food due to the rapid population growth, together with the decline in efficiency of crop production. This phenomenon raises concern for sustainable food production, especially in developing countries. Ferreira (1995) also stressed the important role that biotechnology, which includes micropropagation, could play in the facilitation of the transition to more sustainable agriculture. If an *in vitro* method of propagating elite clones of *Atriplex* species, or individual plants within the same species, can be found it might indirectly contribute to sustainable food production. Bhojwani

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(1990) gave six forces that might influence possible breakthroughs in the future of plant tissue culture. These forces arise from the demands of growers and consumers of plant material.

Table 1 The six forces that might influence possible breakthroughs in plant tissue culture in general and the application to *Atriplex* as fodder species. (Adapted from Bhojwani, 1990)

In general	***Atriplex* as fodder species**
- Product quality, both nutritive and cosmetic	- Nutritive value, palatability
- Lower chemical input requirements in field	- Slower deterioration in homogeneity of *Atriplex* stand
- Lower management input requirements	- Adaptation to poor soil conditions
- Improved post-harvest and transportation characteristic	- Hay production potential
- Pressures of environmental and climatic change	- Pressures of environmental and climatic change
- New product development	- New product development

In Table 1 the six forces, mentioned by Bhojwani (1990), are applied to what might be criteria of importance for selection of *Atriplex* species, or individuals within the same species, as a fodder shrub. New products, for example *Atriplex* cultivars, can be developed by selecting clones with high nutritive value and palatability, which are also adapted to poor soil conditions together with a hay production potential. Refinement of these forces were used as selection criteria in this trial.

Looking just at the above mentioned, *in vitro* propagation of *Atriplex* species might be feasible and play an important role in increasing the use of this species.

There is little published information on micropropagation of *Atriplex* species. Wochok & Sluis (1980) grew *Atriplex canescens in vitro* and tried to develop a method of micropropagation that could eventually be used to produce select *Atriplex* propagules for revegatation programs. Tripathy & Goodin (1989) also tried to establish an efficient method for micropropagation of *Atriplex canescens*. The only information found on micropropagation of *Atriplex nummularia* was on cell suspension cultures that were initiated from callus tissue. Cell growth was measured

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in response to different levels of NaCl to assess the adaption of *A. nummularia* to salinity (Casas, Bressan & Hasegawa, 1991). Koheil, Hilal, El-Alfy & Leistner (1992) reported on the responses of *A. semibaccata* and *A. halimus* to osmotic stress, while grown in cell suspension cultures. *Atriplex halimus* was grown *in vitro* to obtain a homogeneous and salt resistant population and its development while growing *in vitro* was also evaluated at different ratios of Na^{+} and Ca^{++} (Pourrat & Dutuit, 1993). Germination of *Atriplex griffithii* var. *stocksii* was tested *in vitro* at different salinity levels in a liquid medium (Khan & Rizvi, 1994).

Atriplex canescens is the species on which the most information about micropropagation has been collected. The purpose of this trial was to develop efficient methods for micropropagation of *A. nummularia* and *A. canescens* for mass propagation of selected *Atriplex* plants.

MATERIAL AND METHODS

Selection

Plant material were selected at two locations. The first was in the northern Negev, just outside Omer, 7 km. north east of Beer Sheva, in Israel, which receives an average annual rainfall of 120 mm. *Atriplex canescens* and *A. nummularia* plants were selected from a 25 year old introduction trial. These plants had not been grazed by domestic animals and had survived cold winters and droughts. The selection criteria which were used were: survival in cold and drought, green biomass (shoot and leaf) production and an erect growth form, suitable for grazing. The second site was on the Hatfield Experimental Farm of the University of Pretoria, South Africa, which receives an average annual rainfall of 650mm. In contrast to the Omer site, here existing stands of *A. nummularia* were grazed by sheep for six seasons prior to the selection date. The sheep selected certain plants first and those plants were marked as the palatable plants. Eighty plants were initially marked, from which a final selection of 10 was made. The selection criteria were: palatability; leaf retention after the preceding cold winter; good recovery after grazing; and growth form suitable for grazing. The 10 selected plants were propagated vegetatively by rooting cuttings (see Chapter 6). The rooted plants (five of each of the 10 that were selected) were then transferred to five kilogram pots. These pots were placed in a greenhouse to isolate them from the pests and diseases of the natural environment. For the disinfestation and *in vitro*

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culture initiation, cuttings were taken from these greenhouse grown plants for initiation of tissue cultures.

Disinfestation

The first and important step was to find the best method to initiate *in vitro* cultures from wild growing *Atriplex nummularia* plants. Culture initiation was difficult, as plantlets needed to be surface sterilized from possible contaminents, without damaging the plant tissue, to ensure disinfected cultures. According to De Fossard (1986) initiating cultures is a major problem, due to microbial contamination, which can be reduced by growing mother plants in a clean and controlled environment. For that part of the trial, which was conducted in Israel, no mother plants were grown. Cuttings from the selected plants were taken directly from the field and sterilized of microbial diseases, but the success rate was low.

For the trial that was conducted in Pretoria, ten plants were selected in the field as described earlier. Stem cuttings from these plants were rooted and grown in pots in a greenhouse. Tip cuttings were taken from these "mother plants" for disinfestation. The disinfestation of these greenhouse grown mother plants was much more effective.

Israel

Pilot trial

The following procedures were followed. Five centimetre sections were taken from branch tips, washed in a 0.3% liquid detergent (Top Sod) solution for five minutes on a magnetic stirrer and rinsed in running tap water for 30-40 minutes. The cuttings were then washed in a 5% benlate solution for five minutes on a magnetic stirrer. Then from the five centimetre sections only shoot tip- and axillary bud cuttings were taken. These final cuttings were washed in 20% commercial bleach (0.7% sodium hypochlorite) plus 0.1% Tween 20 for five to six minutes on the magnetic stirrer. During the final step the cuttings were rinsed four times in sterile distilled water for eight to ten minutes per rinse, after which the bases of the cuttings were cut away, before putting on agar.

After this pilot trial it was decided to do another disinfestation trial with three different treatments. These were acombination of disinfestation methods described by Jones & van Staden (1994) and Yeoman (1973).

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The following steps of all treatments were the same: Five centimetre sections were taken from branch tips, washed in a 0.3% liquid detergent (Top Sod) solution for five minutes on a magnetic stirrer and rinsed in running tap water for 30-40 minutes. The cuttings were then washed in a 5% benlate solution for five minutes on a magnetic stirrer. Then from the five centimetre sections only shoot tip- and axillary bud cuttings were taken. During the final step the cuttings were rinsed four times in sterile distilled water for eight to ten minutes per rinse, after which the bases of the cuttings were cut away, before putting on agar.

The differences in treatments were as follows:

Treatment 1

The final cuttings were washed in 20% commercial bleach (0.7% sodium hypochlorite) plus 0.1% Tween 20 for five to six minutes on the magnetic stirrer.

Treatment 2

As *Atriplex* species are semi-woody, mercuric chloride ($HgCl_2$) was chosen for one of the treatments. The final cuttings were washed for two to three minutes in a 1.0% $HgCl_2$ solution.

Treatment 3

The final cuttings were washed for two to three minutes in a 1.0% $HgCl_2$ solution. After this the cuttings were washed in 20% commercial bleach (0.7% sodium hypochlorite) plus 0.1% Tween 20 for five to six minutes on the magnetic stirrer.

South Africa

For the work conducted in South Africa another method was tested. As mother plants were grown in a greenhouse, and, therefore, less contaminated than field-grown shrubs, treatment one from the trial in Israel was used with a few modifications. Five centimetre sections were taken from the terminal parts of new shoots. These were washed in a 0.3% liquid detergent (Sunlight Liquid) solution for five minutes on a magnetic stirrer and rinsed in running tap water for 30-40 minutes. After this the cuttings were washed in a 0.5% benlate solution for five minutes on a magnetic stirrer. Then the five centimetre sections were reduced to explant size using only shoot tips and axillary buds. These final cuttings were washed in 20% commercial bleach (0.7% sodium hypochlorite) plus 0.1% Tween 20 for five to six minutes on the magnetic stirrer. During the final step the cuttings were rinsed four times in sterile distilled water for eight to ten minutes per rinse.

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Growth medium and conditions

In the initiation stage sterile explants were established on a basal medium. This medium included: (a) a support material, in this case agar and gelrite; (b) a mineral salt mixture with essential major and minor elements, in this case full strength Murashige and Skoog (MS) basal medium was used (Murashige and Skoog, 1962); (c) an energy source, sucrose; (d) vitamin supplements, in this case nicotinic acid, pyridoxine-HCl, thiamine-HCl (Huang and Murashige, 1976) and myo-inositol(Hartmann, Kester and Davies, 1990); and (e) plant growth regulators, auxins and cytokinins. This basal medium was prepared as in Table 2.

Table 2 The basal medium used throughout the experimental period

Components	Concentration ($g.l^{-1}$)
MS Basal medium	Full strength (Murashige and Skoog, 1962)
Vitamins	($g.l^{-1}$)
nicotinic acid	0.005
pyridoxine-HCl	0.005
thiamine-HCl	0.005
myo-inositol	0.1
pH and gelling agent	($g.l^{-1}$)
pH	5.8
agar	7.5
gelrite	5.0
Energy source	($g.l^{-1}$)
sucrose	30
Plant growth regulators	
Auxin:- NAA	Various concentrations
Cytokinin: - BA	Various concentrations

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The plantlets were grown in test tubes measuring 100 mm by 25 mm. Each test tube contained 10 ml of the growth medium. The test tubes (containing media) were autoclaved for 15 minutes and allowed to set at an angle to minimize contamination of the plantlets. Plant sections (lateral buds and shoot tips) of 4 mm to 6 mm were used for initiation of new cultures or when material was transferred to fresh media. All sterile procedures were conducted in a laminar flow hood. The cultures were placed in a growth room with day length of 16 hours and a constant temperature of 22 °C in Pretoria. In Israel only the temperature differed, being marginally higher at 25 °C. The cultures were allowed to grow for approximately four weeks after which they were transferred to new media.

Following initiation of cultures apparently clean of fungal and bacterial contamination, it was necessary to develop media suitable for shoot multiplication, shoot elongation and rooting. For this, the initiation medium, as described in Table 2, was used, with different auxin and cytokinin concentrations. The auxin that was used was naphthalene acetic acid (NAA) and the cytokinin that was used was 6-benzylaminopurine (BA).

According to Gardner, Pearce & Mitchell (1985), cloning would not be possible without plant growth regulators, such as hormones, which are produced in one part of the plant and function in another part of the plant. It is, therefore, important to add plant growth regulators, because most plant tissues used were detached from their natural source of auxin (Collins, Vian & Phillips, 1978).

Auxins are mainly produced in active meristematic tissues, transported basipetal in the phloem and promote cell elongation, cell division and rooting. Cytokinins are present in young roots and fruits, and transported through the plant via the transpiration stream. Buds are generally a stronger sink for cytokinins than leaves. Cytokinins stimulate cell division, cell and/or shoot differentiation and lateral bud break (Gardner *et al*, 1985).

Adventitious bud formation is stimulated by a synergism between auxins and cytokinin. According to Hartmann *et al* (1990), multiplication of axillary shoots is promoted by controlled inhibition of shoot elongation and stimulation of lateral shoot proliferation, primarily by controlling the concentration and ratio of cytokinin to auxin. To stimulate root formation the auxin level can be increased and the cytokinin concentration decreased or totally left out.

Different concentrations of NAA and BA were tested to develop suitable media for multiplication, shoot elongation and rooting.

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The following measurements were taken of each test tube, at weekly intervals: number and height of shoots; number of leaves; number of buds; callus formation; presence of roots. Shoot heights were measured from the surface of the growth medium to the highest shoot tip. Plant height is important as it shows that the plantlet is growing and also indicates when shoot elongation is taking place. Leave number is also an indication of plant growth as it increases as the plant is growing. The number of buds is an indication of shoot multiplication as new shoots might develop from the buds. The following hypothesis was, therefore, tested: if the height and leaf number increases, the plant is growing; if bud and shoot numbers increase the medium is good for multiplication; if the root number increases the medium is good for rooting and will produce a plantlet ready for transplanting and acclimatization.

Acclimatization

Only the ten *A. nummularia* plants, selected (as described earlier)in South Africa, were used for this trial. Plantlets rooted *in vitro* were selected to be transplanted from *in vitro* to *ex vitro* conditions. Approximately seven plantlets of each of 10 different *A. nummularia* plants were transplanted. The plantlets were taken from the test tubes and the medium was washed from the roots with distilled water, after which the plantlets were planted in pots. Jones & van Staden (1995) washed the growth medium from the roots of *Eucalyptus grandis* with 0.2 % Benlate to reduce fungal contamination. The pots had a capacity of 0.25 kg. and the growing medium was a mixture of sand and peat in a ratio of 1:1. This medium was steam sterilized to reduce fungal contamination of the plantlets.

Factors that should be considered in transplantation are infections and desiccation. According to Withers & Alderson (1986), photosynthesis and transpiration are influenced greatly by the altered leaf structure of cultured plants. The leaves are relatively low in chlorophyll and approximately two weeks after transfer to the greenhouse, the newly formed leaves will show increased photosynthetic activity. Stomata numbers of cultured plants is 50% of that of field grown plants. Excessively high water losses are often recorded from the leaves of plants immediately after transplanting. This water loss is related to: (a) reduced quantities of epicuticular wax; (b) high volume of mesophyll intercellular spaces; and (c) slowness of stomatal response to water stress. Plants were used to high humidity and can get "transplant shock", which leads to tip dieback or death of the plantlets. A period of humidity acclimatization

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is, therefore, required to adapt to the outside environment. During acclimatization the humidity should be gradually reduced over a period of two to three weeks. In this time the plantlets undergo morphological and physiological adaptations enabling them to develop typical terrestrial plant water control. To maintain high humidity a mist spray can be used or the transplanted plants can be covered with a clear plastic or glass cup which are partially tilted to allow air circulation. Hartman *et al* (1990) stressed the importance of keeping the shoot actively growing because the acclimatization and development of autotrophic conditions is dependent on the new growth after transfer from the test tube environment.

With the abovementioned in mind the pots were placed in a glasshouse and covered with a 60% shade cloth. A mist spray was used to maintain high humidity and was controlled with a time switch every five minutes for 10 to 15 seconds. The intervals were gradually increased and after two weeks it was replaced by hand watering twice daily. At the same time the shade cloth was gradually removed by first lifting only the sides and after two weeks it were completely removed. Percentage survival of the plants was assessed four months after transplanting. Plant heights were also measured monthly from the surface of the growth medium to the terminal growth point. Plants with a spindly growth form were pruned back to promote a multi stemmed growth form.

Statistical analyses, where applicable, were conducted with the Kruskal-Wallis partition free one way ANOVA ($p<0.025$) analyses together with the SAS programme (Steel & Torrie, 1980; SAS, 1994). Differences between treatments were tested for measured parameters. Means and standard deviations were measured for each parameter over all treatments.

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RESULTS AND DISCUSSION

Disinfestation

The results of the work done in Israel are illustrated in Table 3. For each treatment 28 cultures were initiated. Treatment one, which was the same as the pilot trial, gave a success rate of only 11% while treatments two and three also gave low levels of success at 36% and 32% respectively.

Table 3 Survival rate of *Atriplex nummularia* plants at several stages after disinfestation

	Treatment					
	1		2		3	
Days after initiation	0.7% sodium hypochlorite		1.0% $HgCl_2$		0.7% sodium hypochlorite plus 1.0% $HgCl_2$	
	clean	contaminated	clean	contaminated	clean	contaminated
	(%)		(%)		(%)	
4	25	75	79	21	64	36
7	11	89	57	43	46	54
9	11	89	43	57	32	68
10	11	89	36	64	32	68
Average percentage clean cultures for all 3 treatments:				26		

These rather poor results could be related to the fact that field grown mother plants were used and not greenhouse grown mother plants as was suggested by De Fossard (1986). Another explanation could have been too high a concentration of the disinfectants: sodium hypochlorite (NaOCl); $HgCl_2$ or Benlate. According to Hughes (1981) the correct concentrations of these disinfectants and time of exposure should be tested for and used, for each plant species and each explant type. The explants in some of the test tubes turned yellow and died despite not being contaminated, which is an indication of damage to living tissue, probably due to harsh disinfectant treatment. The time of exposure to the disinfectants could also have played a role. After this trial, disinfestation of five *Atriplex canescens* and five *Atriplex nummularia* plants was conducted by using treatment two (benlate and $HgCl_2$ as disinfectants). The explants were planted on two different media. The one medium (treatment 1) consist only of the Murashige

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and Skoog (MS) basal medium with vitamins and no hormones, while the other medium (treatment 2) was the same medium with the addition of 0.20 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA. The results are presented in Tables 4 and 5. No disinfestation method for *A. canescens* was tested and the method developed for *A. nummularia* was used. This might explain the poor results with an average of only 21% developing (disease free) cultures. Plant numbers four and six had an average of only 7.5% clean, developing cultures, which was much lower than the 30% of plant numbers three, five and 10. This might, however, be due to the large genetic variation between different plants within this genus, as are mentioned by Senock, Barrow, Gibbens & Herbel (1991).

Table 4 Results (average percentage) of the disinfestation of five different *Atriplex canescens* plants. Twenty cultures were initiated per plant per treatment. The explants were planted on two different media, treatments one and two.

Treatment no.	Plant no.	contaminated	clean but dead	developing	Plant no.	developing (%) average of treatments 1 and 2
		%				
1	3	35.0	55.0	10.0	3	30.0
0.00 $mg.l^{-1}$	4	65.0	35.0	0.0	4	7.5
BA	5	55.0	25.0	20.0	5	30.0
0.00 $mg.l^{-1}$	6	50.0	50.0	0.0	6	7.5
NAA	10	35.0	30.0	35.0	10	30.0
average		48.0	39.0	13.0	average	21.0
2	3	20.0	30.0	50.0		
0.20 $mg.l^{-1}$	4	50.0	35.0	15.0		
BA	5	20.0	40.0	40.0		
0.05 $mg.l^{-1}$	6	30.0	55.0	15.0		
NAA	10	50.0	25.0	25.0		
average		34.0	37.0	29.0		

The disinfestation results of the five *A. nummularia* plants are illustrated in Table 5. The average for all plants over both treatments was rather low at 36.5%. Concerning the five different plants, there were once again differences, with plant numbers one and seven having a low percentage developing cultures of 25% and 15% respectively while plant numbers two, eight and nine had higher figures of 45%, 52.5% and 45% respectively. Treatment one had 45% healthy cultures in comparison with the 28% of treatment 2.

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Table 5 Results (average percentage) of the disinfestation of five different *Atriplex nummularia* plants. Twenty cultures were initiated per plant per treatment. The explants were planted on two different media, treatments one and two.

Treatment no.	Plant no.	contaminated	clean but dead	developing	Plant no.	developing (%) average of treatments 1 and 2
			%			
1	1	5.0	65.0	30.0	1	25.0
0.00 mg.l^{-1}	2	0.0	45.0	55.0	2	45.0
BA	7	25.0	60.0	15.0	7	15.0
0.00 mg.l^{-1}	8	5.0	20.0	75.0	8	52.5
NAA	9	5.0	45.0	50.0	9	45.0
average		8.0	47.0	45.0	average	36.5
2	1	30.0	50.0	20.0		
0.20 mg.l^{-1}	2	20.0	45.0	35.0		
BA	7	15.0	70.0	15.0		
0.05 mg.l^{-1}	8	15.0	55.0	30.0		
NAA	9	30.0	30.0	40.0		
average		22.0	50.0	28.0		

The influence of the two different media, will be discussed later, as there are too many factors playing a role during the disinfestation process, which might have influenced the outcome of the disinfestation process.

In South Africa the method of disinfestation was slightly changed, as described earlier, and these results are illustrated in Table 6. One of the differences between this and the other trial was that 10 different *A. nummularia* plants were used.

Table 6 Average percentage survival rate of 10 different *Atriplex nummularia* plants 21 days after disinfestation. Fifteen cultures were initiated per plant.

Plant no.	clean	contaminated
	%	
1	88	12
2	78	22
3	76	24
4	79	21
5	83	17
6	100	0
7	75	25
8	78	22
9	92	8
10	71	29
avg	82	18

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The percentage clean cultures ranged from 71 - 100% with an average of 82%. This is much higher than the average values of the trials in Israel which ranged form 21% to 36%. This high success rate might be explained in two ways. Firstly, mother plants were grown in a controlled environment in a greenhouse, where the plants were not exposed to contaminants like bacteria and fungi. No overhead irrigation was used and that might also have helped to keep the new growth of the mother plants away from contact with contaminants. Secondly, no $HgCl_2$ was used and a lower percentage (0.5% instead of 5.0%) of Benlate was used. It was possible to use this weaker disinfectant treatment as the mother plants were grown in a relatively contaminant free environment.

According to Hartman *et al* (1990) the effectiveness of bleach (potassium hypochlorite) treatment is a time dosage response; the disinfestation effectiveness increases with an increase in both time and concentration, but the damage to living tissue also increases. Thus a compromise must be developed for the kind of explant through testing.

In this work concentration and exposure time was based on previous experience with other species. It is, therefore, suggested that even higher success rates might be achieved if different dosages, regarding concentrations and time, of bleach, are tested. If it is unavoidable to use mother plants directly from the field, Hughes (1981) suggests that tips of newly sprouted shoots should be used to get a high disinfestation success rate. It is also suggested that three centimetre shoot pieces could be grown in a liquid medium and cuttings from their new growth can be grown on a agar medium. Such a liquid medium should be without sugar, which would considerably reduce the growth of contaminants.

Culture growing: *A. canescens*

After disinfestation it was necessary to identify media suitable for multiplication, shoot elongation and rooting. The disinfected cultures were transferred to media containing different concentrations of BA and NAA. Several trials were conducted and the results are illustrated in Tables 7, 8, 9, 10 and 11 as the average values for different amounts of cultures.

Pilot trial

A pilot trial was conducted with *A. canescens* together with the first disinfestation. Two treatments were used, namely the basal medium (see Table 2) without any plant growth

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regulators, while the second treatment was the basal medium with 0.20 mg.l^{-1} BA and 0.05 mg.l^{-1} NAA. *Atriplex canescens* had a higher survival rate on the medium with hormones than the medium without hormones (Table 7).

Table 7 Average percentage survival of *A. canescens* with two different treatments in the pilot trial. Twenty cultures were evaluated per treatment.

Treatment	BA (mg.l^{-1})	NAA (mg.l^{-1})	% Survival
1	0.00	0.00	13
2	0.20	0.05	29

The cultures from treatment two had more leaves, axillary buds and callus tissue than the cultures from treatment one. The survival rates (after disinfestation) were, however, low and thus the numbers of cultures surviving were also very low. It is, therefore, dangerous to draw final conclusions from these results.

This trial was started with five different *A. canescens* plants. The clones generated from these plants were mixed during the course of the trial. The results of *A. canescens* will, therefore, be an average of the total batch with no definite figures for specific plants. The contribution of each of the five plants to the average values is, therefore, unknown.

Trial 1

The results for the first trial after the pilot trial are illustrated in Table 8. Treatment one of the pilot trial (no hormones) was left out because of its poor results, but this might have been a mistake as the number of cultures that were evaluated was very low and not truly representative. Three treatments were used as illustrated in Table 8.

Table 8 Averages values for the different parameters of Trial 1 of *A. canescens* nine days after culture initiation. Twelve cultures were evaluated per treatment

Treatment no.	BA mg.l^{-1}	NAA mg.l^{-1}	Shoot height(mm)	Shoots no.	Buds no.	Callus %
1	0.20	0.05	13.3	1.0	4.0	100.0
2	0.20	0.50	10.4	0.0	3.8	100.0
3	1.00	0.05	10.0	0.0	1.9	100.0
mean			11.3 ± 4.7	0.3 ± 1.4	3.3 ± 1.8	

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According to the statistical analyses the only significant ($p<0.025$) difference was the average number of buds in treatment one (4.0), which were significantly higher than the 1.9 buds of treatment three. Overall, treatment one proved to be the best treatment as it gave the best growth with respect to plant height and number of buds. In this trial, treatment one was the best for shoot elongation with the highest average plant height of 13.3 mm, and the best for multiplication with the highest average number of buds (4.0). No root formation was recorded for any of the treatments. Callus formation was recorded in all cultures of all three treatments.

Trial 2

The cultures from Trial 1 were subcultured on fresh media in Trial 2. The same three treatments were used and measurements were taken once weekly over a four week period. The results are illustrated in Table 9. The only significant ($p<0.025$) differences were between the heights of treatment one and two for weeks three and four. By week four, the average plant height for treatments one and two were 4.5 mm and 17 mm respectively.

Table 9 Average values for the different parameters of Trial 2 of *A. canescens* over a four week period. Six cultures were evaluated per treatment

Treatment	BA	NAA	Height (mm)				Number of shoots			
no.	mg.l^{-1}	mg.l^{-1}	week1	week2	week3	week4	week1	week2	week3	week4
1	0.20	0.05	4.3	4.5	4.5	4.5	1.0	1.0	1.0	1.0
2	0.20	0.50	9.1	10.9	13.1	17.0	1.0	1.0	1.0	2.6
3	1.00	0.05	6.9	8.1	9.4	11.1	1.0	1.0	1.1	1.3
mean			7.3±3.9	8.5±4.2	9.9±5.1	12.2±7.9	1.0±0.0	1.0±0.0	1.1±0.2	1.8±3.0

Treatment	Number of leaves				Number of buds				Callus (%)			
no.	week1	week2	week3	week4	week1	week2	week3	week4	week1	week2	week3	week4
1	5.8	5.8	7.0	7.5	0.8	0.8	1.0	1.3	0	25	50	50
2	6.4	10.5	15.0	19.3	1.1	3.0	4.3	5.4	0	25	63	75
3	7.6	11.0	13.0	15.3	1.6	2.3	2.9	3.9	28	28	57	71
mean	6.7±4.1	9.7±5.3	12.6±7.8	15.3±10.3	1.2±1.4	2.3±2.0	3.1±2.9	3.9±3.7				

Concerning the fact that a medium is needed for each of the following: shoot multiplication, shoot elongation and rooting, the following suggestions are made. Treatment two will be good for shoot multiplication and elongation, as its cultures had the highest average height (17 mm), number of buds (5.4) and number of shoots (2.6) after four weeks. Plantlets from treatment one did not grow as well as in Trial 1. The plants were very small and formed only one shoot and

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one bud with very few leaves, which might be a result of the type of cutting that was transferred from Trial 1. Most of the cuttings for this trial were a piece of shoot with one axillary bud. These cuttings tended not to develop into plants, while shoot tip cuttings tended to develop into plants more easily. Another reason might be the low auxin level of only 0.05 $mg.l^{-1}$ NAA, as this stimulates elongation and restores apically dominant growth (de Fossard, 1986). Higher auxin levels might result in the development of a new plant from the axillary bud.

Trial 3

For this trial the cultures of Trial 2 were transferred to fresh media and the same three treatments were used. Measurements were taken over the first two weeks and again at the end of the sixth week. High levels of vitrification occurred at all three treatments and no statistical analyses were therefore possible. The results are illustrated in Table 10. As in Trial 1, treatment one (0.20 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA) proved to be the best treatment. The average plant height of treatment one was 39.7 mm after six weeks, which was much higher than that of treatment two and three, at 8.3 mm and 12.3 mm respectively. Treatment one also formed more shoots, leaves and buds than the other two treatments.

After two weeks 6% of the cultures of treatment one exhibited root formation and after six weeks 53%.

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Table 10 Average values for the different parameters of Trial 3 of *A. canescens* 1 week, 2 weeks and 6 weeks after culture initiation. Ten cultures were evaluated per treatment

Treatment	BA	NAA	Height(mm)			Number of shoots			Number of leaves			Number of buds		
no.	mg.l^{-1}	mg.l^{-1}	week1	week2	week6	week1	week2	week6	week1	week2	week6	week1	week2	week6
1	0.20	0.05	14.9	16.6	39.7	1.1	2.5	3.6	12.6	16.6	23.6	3.6	5.2	7.3
2	0.20	0.50	7.5	7.5	8.3	1.0	1.0	1.0	8.5	8.8	9.5	0.0	0.5	0.5
3	1.00	0.05	11.3	12.3	12.3	1.0	2.3	1.0	10.3	12.3	10.5	1.3	3.0	2.0

Treatment	BA	NAA	Callus (%)			Vitrification (%)			Roots (%)		
no.	mg.l^{-1}	mg.l^{-1}	week1	week2	week6	week1	week2	week6	week1	week2	week6
1	0.20	0.05	96.6	96.6	96.6	41.0	41.0	41.0	0.0	6.0	53.0
2	0.20	0.50	94.7	94.7	94.7	82.6	82.6	82.6	0.0	0.0	0.0
3	1.00	0.05	87.5	87.5	87.5	72.7	72.7	72.7	0.0	0.0	0.0

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A photo of one of the *A. canescens* cultures from treatment one (0.20 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA) can be seen in Figure 1. In this picture the height, multiple shoots, many leaves and some roots can be seen, just as illustrated in treatment one in Table 10. None of the cultures of the other two treatments had roots. This was, however, not expected as higher levels of auxin, such as the 0.50 $mg.l^{-1}$ NAA in treatment two, were expected to enhance root formation rather than the 0.05 $mg.l^{-1}$ NAA of treatments one and three. Treatment one appeared to be a better medium for multiplication, shoot elongation and rooting, than the other treatments. Treatment one also had the lowest percentage of cultures showing vitrification, of only 41%, while treatments two and three had values of 82.6% and 72.7% respectively.

Vitrification was also visible in the first two trials but not to this extent. A good description of vitrification was given by Hartman *et al* (1990) who said that the cultures have a water soaked, succulent appearance that can result in a failure to proliferate. It can be physiologically expressed that water uptake is excessive and inhibition of lignin and cellulose synthesis occurs. These disorders, which are manifested mainly in the leaves, affect two major processes carried out by the leaves, namely: photosynthesis and gas exchange. The stems and roots are also affected but to a lesser extend (Ziv, 1990). This appears to be a consequence of the matric potential of the medium as well was a low nitrate - ammonium relationship (Hartman *et al*, 1990). According to Ziv (1991) relative humidity and the water potential are the key factors in this abnormal morphogenesis *in vitro*. According to Hartmann *et al* (1990) vitrification is most prevalent if plants are grown in medium with low agar concentration and high ammonium concentrations as is the case with the MS medium, which was used in this trial. To overcome this problem more agar can be used, or a lower strength MS medium, or modification to the BA concentration or combinations of these can be tested in the future. Tripathy and Goodin (1989) mentioned that the ½-strength MS salts were optimal for shoot growth of *A. canescens*, because in their study full strength MS had never produced shoots or shoot buds. They never mentioned anything about vitrification, which might be the result of only using ½-strength MS salts.

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Figure 1 An example of an *A. canescens* plant, growing *in vitro* on medium (treatment) number one (0.20 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA)

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Trial 4

The cultures from Trial 3 were divided and transferred to fresh media. Six different treatments, based on different concentrations of BA and NAA, were used. Treatments one and two were the same as treatment one and two of the previous two trials. Treatment three from the previous trials, with 1.0 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA, was left out as no meaningful culture growth and development took place on this medium. Measurements were taken over the first three weeks and again after seven weeks. Statistical analyses did not include week seven, % callus and % roots. Results are illustrated in Table 11. The only significant ($p<0.025$) differences were found between the number of shoots for week two. Average number of shoots (1.4) for treatments six, was significantly higher than the 1.0 for treatments three and five. This information is, however, of no significance as the opposite was true for weeks three and seven, where treatments three and five had higher shoot numbers than treatment six. The best medium for multiplication appeared to be treatment three, as it had the highest average number of shoots (10), the highest average number of leaves (24) and together with treatment four the highest number of buds (eight), seven weeks after culture initiation.

Treatment six (0.05 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA), might be the best medium for shoot elongation as it produced an average shoot height of 74 mm after seven weeks, which was much higher than any of the other treatments. For both treatments three and six, 100 % of the cultures formed roots after seven weeks. It would be suggested that treatment six is a better medium for rooting as it formed fewer shoots, (an average of only 2.5), than treatment three and also formed elongated shoots, more than twice the length of treatment three.

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Table 11 Average values for the different parameters of Trial 4 for *A. canescens* 1 week, 2 weeks, 3 weeks and 7 weeks after culture initiation. Ten cultures were evaluated per treatment

Treatment	BA	NAA	Height (mm)				Number of shoots				Number of leaves			
no.	mg.l^{-1}	mg.l^{-1}	week1	week2	week3	week7	week1	week2	week3	week7	week1	week2	week3	week7
1	0.20	0.05	14.1	16.4	20.9	nm	1.0	1.0	1.6	nm	6.1	10.6	15.1	nm
2	0.20	0.50	12.5	15.4	18.6	28.5	1.3	1.6	2.7	5.8	6.8	11.2	14.9	20.8
3	0.20	1.00	13.8	15.1	16.5	27.0	1.0	1.0	2.5	10.0	5.5	11.4	15.2	24.0
4	0.20	0.10	14.4	17.1	22.4	28.0	1.0	1.0	3.3	8.0	5.1	10.4	15.9	21.0
5	0.50	0.05	16.1	17.7	20.1	38.7	1.0	1.0	2.8	4.5	6.4	12.4	15.3	20.7
6	0.05	0.05	17.3	21.3	25.7	74.0	1.0	1.4	1.6	2.5	5.4	10.6	15.0	20.0
mean			15.1±6.0	17.6±7.2	21.2±10.3		1.1±0.4	1.2±0.9	2.7±2.4		6.2±2.2	11.5±2.8	15.8±2.4	

Treatment	BA	NAA	Number of buds				Callus (%)				Roots (%)			
no.	mg.l^{-1}	mg.l^{-1}	week1	week2	week3	week7	week1	week2	week3	week7	week1	week2	week3	week7
1	0.20	0.05	1.0	2.0	3.3	nm	0.0	14.0	100.0	nm	0.0	0.0	0.0	nm
2	0.20	0.50	1.0	2.6	3.9	6.5	18.0	82.0	100.0	100.0	0.0	0.0	0.0	25.0
3	0.20	1.00	1.0	2.7	3.9	8.0	0.0	75.0	92.0	100.0	0.0	0.0	0.0	100.0
4	0.20	0.10	1.0	2.3	3.6	8.0	0.0	43.0	100.0	100.0	0.0	0.0	0.0	0.0
5	0.50	0.05	1.0	3.6	4.3	5.7	0.0	33.0	92.0	92.0	0.0	0.0	0.0	0.0
6	0.05	0.05	0.0	3.0	4.4	3.0	0.0	14.0	100.0	100.0	0.0	0.0	0.0	100.0
mean			0.3±0.5	2.4±1.6	4.0±1.8									

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Something of concern in Trials 3 and 4 was the fact that no major differences between treatments were visible during the first three weeks after culture initiation. At six and seven weeks more meaningful differences were witnessed between the treatments. A seven week incubation period might be too long (Hartmann *et al*, 1990) and attempts should be made to enhance culture development to make this procedure economically viable for the mass production of elite material over a short time period.

Wochok & Sluis (1980) found that multiple buds produced *in vitro* for *A. canescens*, generally remained in a rosette condition. This was also experienced in this experiment with *A. canescens*. The only medium with good shoot elongation was in treatment six of Trial 4 with 0.05 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA. Both Wochok & Sluis (1980) and Tripathy & Goodin (1989) obtained sufficient shoot elongation of *A. canescens* by adding gibberellic acid in the GA_3 form to their growth media. For these researchers a good height was 10 to 15 mm as they were using petri dishes, while for this trial test tubes were used and heights of up to 70 mm were registered. On the other hand other authors took measurements only up to 14 days (two weeks) and shoot heights from this trial were more or less the same after two weeks as those of Wochok & Sluis (1980) and Tripathy & Goodin (1989). Wochok & Sluis (1980) also found three different combinations of BA and NAA that had the ability to sustain regeneration capacity for *A. canescens*. One of them was the same as treatment five in Trial 4 with 0.5 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA. This treatment in Trial 4 was also acceptable, as it showed sufficient shoot formation and elongation. None of the previously mentioned authors finished their rooting trials before publishing their data on *A. canescens*. Although 100 % rooting was found in two of the treatments in Trial 4, this was not yet satisfactory as it took seven weeks to develop. To induce root formation very low levels, or no cytokinin and higher auxin levels should be applied (Hartman *et al*, 1990). This was a shortcoming of this experiment as low levels of cytokinin were not tested together with high auxin levels or auxins alone were also not tested. This should be done in the future.

From this experiment it can be concluded that the basal medium with the addition of 0.20 $mg.l^{-1}$ BA and 1.0 $mg.l^{-1}$ NAA was good for shoot multiplication and elongation while a medium with 0.05 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA was good for elongation and rooting of *A. canescens*. It is also important to remember the genetic variation within *A. canescens*, between different plants of this one species, may have an influence on results as were reported by Wochok & Sluis (1980) and Tripathy & Goodin (1989).

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Culture growing: *A. nummularia*

For the first pilot trial only one plant of *A. nummularia* was used. After disinfestation the material that survived was transferred to fresh media with two treatments. One treatment was the basal medium without any plant growth regulators, while the second treatment contained 0.20 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA. Only four cultures for treatment one and eight for treatment two were initiated. The cuttings, mainly axillary buds, were, however, too small and did not grow. The cultures from treatment one outlived those from treatment two but after some weeks all cultures died. This might have been due to the transferred plantlets being too small. In follow up trials five different *A. nummularia* plants were selected, disinfected and cultured. These five plants were each given a number and they were kept separated from each other to see if they reacted differently when cultured *in vitro*. Four trials were conducted and each is described separately.

Trial 1

After the disinfestation trial, where five new *A. nummularia* plants were cultured *in vitro*, the cultures were divided and transferred to fresh media in three treatments. The basal medium remained the same as described in Table 2, with only the concentration of BA and NAA being varied. The treatments and results of Trial 1 are presented in Table 12.

Table 12 Average values for the measured parameters of Trial 1 for five different *A. nummularia* plants, 10 days after culture initiation. Five cultures were evaluated per plant per treatment.

Treatment no.	BA $mg.l^{-1}$	NAA $mg.l^{-1}$	Plant no.	Shoot height	Shoots no.	Buds no.	Callus %
1	0.00	0.00	1	6.4	1.0	1.6	80.0
1	0.00	0.00	2	7.6	1.0	1.6	20.0
1	0.00	0.00	7	20.5	1.0	1.0	50.0
1	0.00	0.00	8	24.3	2.6	1.1	12.5
1	0.00	0.00	9	8.3	2.2	2.2	0.0
			mean	13.4 ± 8.3	1.6 ± 0.8	1.5 ± 0.5	32.5
2	0.20	0.05	1	4.0	1.0	2.0	50.0
2	0.20	0.05	2	9.5	3.6	2.9	25.0
2	0.20	0.05	7	22.5	8.0	4.5	0.0
2	0.20	0.05	8	25.3	2.8	1.5	12.5
2	0.20	0.05	9	9.7	2.2	1.8	16.6
			mean	14.2 ± 9.2	3.5 ± 2.7	2.5 ± 1.2	20.8
3	0.50	0.05	1	11.5	1.0	1.0	50.0
3	0.50	0.05	2	12.3	4.0	2.7	28.6
3	0.50	0.05	7	16.7	5.7	3.7	0.0
3	0.50	0.05	8	17.4	1.0	0.9	28.6
3	0.50	0.05	9	9.7	2.0	2.0	14.3
			mean	13.5 ± 3.4	2.7± 2.0	2.0 ± 1.2	24.3

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The results are given at only one time period after initiation (10 days). The data were not balanced with, for example, only two cultures for plant number one per treatment, while plant number eight had six cultures per treatment. Average plant heights were virtually the same for all three treatments, although plants number seven and eight produced taller shoots than the other three plants on treatments one and two. Treatment two produced more shoots and buds than the other two treatments, which will make it a better multiplication medium. This was unexpected as treatment three had a higher cytokinin concentration and was theoretically supposed to give higher shoot and bud numbers than the other two treatments. The percentage of cultures with callus formation did not vary much between the three treatments, while plant number one tended to exhibit higher callus formation in all three treatments than the other four plants.
It is difficult to compare five such plants, because they have some genetical differences even though they are from the same species. Differences between plants can largely be described within treatments rather than between different treatments.

Trial 2

Plant material from Trial 1 were transferred to fresh media for Trial 2. In this trial the same three treatments were used and measurements were taken once a week over a four week period. Statistical analyses were done for plants numbers eight and nine only and for all measurements except the percentage callus and roots. No statistically significant ($p<0.025$) differences were found. The data is presented in Table 13. The percentage callus and percentage roots indicates the percentage of test tubes (cultures) that had callus and root formation. Treatment one had an average plant height of 29.8 mm after four weeks which were much higher than the 15.7 mm and 8.7 mm of treatments two and three respectively, after four weeks. Treatment two had a higher average number of shoots, leaves and buds for each of the four weeks than either of the other treatments, which will make it the best multiplication medium. This is in line with Trial 1 where treatment two was also the better medium for multiplication. Treatment one, which is the basal medium without any plant growth regulators, shows the highest average plant height for all four weeks and is also, with the exception of plant number two, the only treatment where root initiation occurred. Treatment one was, therefore, better for both elongation and rooting than treatments two and three. Plant number two had no root formation while plant number seven had 100 percent root formation. Looking at all the measurements plant number seven appeared to be better adapted to *in vitro* cultivation than the other four plants.

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Table 13 Average values for measured parameters of Trial 2 for *A. nummularia* at four weeks after culture initiation. Date that were not measured are indicated as: nm. Five cultures were evaluated per plant per treatment

Treatment	BA	NAA	Plant	Height(mm)				Shoots				Number of leaves			
no.	mg/l	mg/l	no.	week1	week2	week3	week4	week1	week2	week3	week4	week1	week2	week3	week4
1	0.00	0.00	1	24.0	29.0	37.5	53.5	1.0	1.0	1.0	1.0	6.5	7.0	11.0	12.5
1	0.00	0.00	2	13.0	14.5	16.9	8.5	1.3	1.3	1.4	2.5	6.8	8.5	11.1	13.0
1	0.00	0.00	7	16.0	19.0	30.0	44.0	1.0	1.0	1.0	1.0	5.5	11.5	14.5	21.5
1	0.00	0.00	8	17.8	20.1	22.1	25.3	1.0	1.0	1.0	1.0	4.7	6.7	9.4	10.1
1	0.00	0.00	9	12.6	14.8	15.6	17.8	1.0	1.0	1.0	1.0	6.5	6.6	8.9	9.6
			mean	16.7	19.5	24.4	29.8	1.1	1.1	1.1	1.3	6.0	8.1	11.0	13.3
2	0.20	0.05	1	10.0	16.0	16.0	nm	1.0	4.0	8.0	nm	13.0	16.0	17.0	nm
2	0.20	0.05	2	8.4	11.0	13.4	nm	1.3	1.6	2.0	nm	6.4	8.7	10.6	nm
2	0.20	0.05	7	12.5	15.7	20.9	27.9	1.7	1.3	1.8	2.7	5.9	9.0	13.1	18.3
2	0.20	0.05	8	19.1	20.9	23.4	28.9	1.1	1.1	1.1	1.0	5.3	6.7	9.8	9.4
2	0.20	0.05	9	11.8	14.3	17.8	21.8	1.5	1.7	1.8	2.7	5.9	8.3	11.3	14.7
			mean	12.4	15.6	18.3	15.7	1.3	1.9	2.9	1.3	7.3	9.7	12.4	8.5
3	0.50	0.05	1	14.8	17.3	22.5	nm	1.0	1.3	1.3	nm	5.0	7.3	10.0	nm
3	0.50	0.05	2	11.3	13.1	15.6	nm	1.6	2.3	2.2	nm	6.2	9.3	10.3	nm
3	0.50	0.05	7	11.4	14.3	17.7	nm	1.0	1.0	2.4	nm	5.9	10.2	13.9	nm
3	0.50	0.05	8	16.1	18.8	20.9	24.1	1.0	1.0	1.0	1.0	4.7	6.6	9.0	10.7
3	0.50	0.05	9	13.4	16.1	17.6	19.3	1.0	1.0	1.0	1.0	5.1	8.1	9.9	13.0
			mean	13.4	15.9	18.9	8.7	1.1	1.3	1.6	0.4	5.4	8.3	10.6	4.7

Treatment	BA	NAA	Plant	Number of buds				Callus (%)				Roots (%)			
no.	mg/l	mg/l	no.	week1	week2	week3	week4	week1	week2	week3	week4	week1	week2	week3	week4
1	0.00	0.00	1	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	50.0	50.0	50.0
1	0.00	0.00	2	1.7	1.8	2.1	3.0	0.0	10.0	30.0	30.0	0.0	0.0	0.0	0.0
1	0.00	0.00	7	3.0	4.0	5.0	4.5	0.0	0.0	50.0	50.0	0.0	50.0	50.0	100.0
1	0.00	0.00	8	1.5	2.2	2.0	1.8	0.0	0.0	21.4	28.6	0.0	0.0	7.1	7.1
1	0.00	0.00	9	2.0	2.3	2.6	2.6	0.0	0.0	100.0	100.0	0.0	0.0	12.5	12.5
			mean	1.8	2.2	2.5	2.6	0.0	2.0	40.3	41.7	0.0	20.0	23.9	33.9
2	0.20	0.05	1	4.0	8.0	10.0	nm	0.0	0.0	100.0	nm	0.0	0.0	0.0	nm
2	0.20	0.05	2	1.9	3.1	3.6	nm	14.3	42.9	92.9	nm	0.0	0.0	0.0	nm
2	0.20	0.05	7	1.9	3.0	4.8	4.7	11.5	73.1	100.0	100.0	0.0	0.0	0.0	0.0
2	0.20	0.05	8	1.6	2.0	2.2	2.0	26.3	57.9	78.9	90.0	0.0	0.0	0.0	0.0
2	0.20	0.05	9	1.8	2.7	3.8	5.2	25.0	75.0	100.0	100.0	0.0	0.0	0.0	0.0
			mean	2.3	3.7	4.9	2.4	15.4	49.8	94.4	58.0	0.0	0.0	0.0	0.0
3	0.50	0.05	1	1.0	1.0	1.8	nm	0.0	50.0	100.0	nm	0.0	0.0	0.0	nm
3	0.50	0.05	2	2.5	3.6	4.5	nm	0.0	50.0	90.0	nm	0.0	0.0	0.0	nm
3	0.50	0.05	7	2.1	2.9	4.2	nm	16.7	83.3	100.0	nm	0.0	0.0	0.0	nm
3	0.50	0.05	8	1.4	1.8	1.9	1.7	23.5	64.7	76.5	80.0	0.0	0.0	0.0	0.0
3	0.50	0.05	9	2.1	2.0	2.3	2.8	20.0	93.3	100.0	100.0	0.0	0.0	0.0	0.0
			mean	1.8	2.3	2.9	0.9	12.0	68.3	93.3	36.0	0.0	0.0	0.0	0.0

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Trial 3

For the next transfer from Trial 2 to Trial 3 it was decided to use six different treatments. Treatment one and two were the same as in the previous two trials and treatment three of the previous two trials was left out as the results did not warrant continuation. The new treatments were mainly combinations of high BA (cytokinin) concentrations and low NAA (auxin) concentrations and can be seen in Table 14. Only plant numbers seven, eight and nine were used, as the survival rate of plant numbers one and two were not high enough to produce enough plant material. Measurements were taken at two, three and four weeks after culture initiation.

Results of Trial 3 are illustrated in Table 14. Statistical analyses were done for all data, with the exception of root and callus percentages. The only significant ($p<0.025$) differences were between number of leaves of plant seven and eight. For weeks three and four and for all six treatments the number of leaves for plant seven were significantly (<0.025) higher than that of plant eight. Therefore, plant number seven is better adapted to *in vitro* propagation than plant eight as it grew much better. This indicates the genetic differences between different plants from the same species.

Sixty percent of the cultures of plant number seven formed roots in treatment one and three, while 60 percent of the cultures of plant nine formed roots in treatment one. Plant eight never formed roots and none of the other treatments (three to six) induced root formation. For the other measurements, no important differences were visible between plants.

Plant nine is illustrated in Figure 2 for the six different treatments. Although the picture is not very clear, it is clear that shoot height and root formation of treatment one is higher than the other five treatments. From Figure 2 it is also visible that the culture growing on medium (treatment) three was the weakest of the six treatments. This can also be seen by the low shoot length, number of shoots and leaf number for treatment three of plant nine in Table 14.

Treatment one once again gave the highest average plant height of 41.6 mm with treatment four the lowest at 27.0 millimeters. Treatment one was again the best medium for shoot elongation and rooting, as it also had the highest average percentage of cultures with root formation; forty percent in comparison with the 20% for treatment three and none for the other treatments. Looking at the number of shoots, leafs and buds, there are no significant differences between the treatments. Treatment two (0.20mg.l^{-1} BA and 0.05 mg.l^{-1} NAA), however, produced the highest number of buds and leafs and might, therefore, be the best medium for multiplication.

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Table 14 Average values of the measured parameters of Trial 3 for three different *A. nummularia* plants, at week 2, week 3 and week 4 after culture initiation. Five cultures were evaluated per plant per treatment.

Treatment	BA	NAA	Plant	Height (mm)			Number of shoots			Number of leaves			Number of buds			Callus (%)			Roots (%)		
no.	$mg.l^{-1}$	$mg.l^{-1}$	no.	week2	week3	week4	week2	week3	week4	week2	week3	week4	week2	week3	week4	week2	week3	week4	week2	week3	week4
1	0.00	0.00	7	29.8	38.8	44.6	1.0	1.0	1.4	10.4	13.2	17.4	2.0	2.2	2.2	40.0	40.0	60.0	40.0	60.0	60.0
1	0.00	0.00	8	29.8	33.2	34.8	1.0	1.2	1.2	7.8	9.2	12.4	1.4	1.2	1.4	0.0	0.0	0.0	0.0	0.0	0.0
1	0.00	0.00	9	20.2	28.6	45.4	1.0	1.0	1.0	11.8	14.4	19.4	2.2	2.6	4.6	40.0	60.0	40.0	60.0	60.0	60.0
			mean	26.6±5.5	33.5±5.1	41.6±5.9	1.0±0.0	1.1±0.1	1.2 ±0.2	10.0±2.0	12.3 ±2.7	16.4±3.6	1.9±0.4	2.0±0.7	2.7±1.7	26.7	33.3	33.3	33.3	40.0	40.0
2	0.20	0.05	7	34.5	39.0	53.8	2.0	3.0	3.0	13.5	14.0	19.3	3.5	4.3	4.3	100.0	100.0	100.0	0.0	0.0	0.0
2	0.20	0.05	8	26.8	30.4	29.6	1.2	1.2	1.2	11.0	12.6	16.4	2.8	3.4	3.8	40.0	40.0	60.0	0.0	0.0	0.0
2	0.20	0.05	9	22.8	25.4	30.8	1.0	1.0	1.0	12.0	14.0	16.2	4.2	4.4	4.2	100.0	100.0	100.0	0.0	0.0	0.0
			mean	27.6±5.3	30.9±5.8	35.7±9.5	1.5±0.7	1.9±1.5	1.9±1.5	12.1±1.2	13.4±0.7	17.2±1.6	3.4±0.7	4.0±0.6	4.1±0.3	80.0	80.0	86.7	0.0	0.0	0.0
3	0.00	0.05	7	19.2	21.4	33.8	1.0	1.0	1.0	8.6	13.4	17.8	1.6	2.0	2.8	0.0	80.0	80.0	0.0	20.0	60.0
3	0.00	0.05	8	22.0	23.6	26.0	1.0	1.0	1.0	6.6	8.2	9.8	1.2	1.4	1.6	0.0	0.0	0.0	0.0	0.0	0.0
3	0.00	0.05	9	17.8	18.6	21.8	1.0	1.0	1.0	7.4	9.2	10.2	1.2	1.4	1.4	0.0	0.0	40.0	0.0	0.0	0.0
			mean	19.7±2.1	21.2±2.5	27.2±6.1	1.0±0.0	1.0±0.0	1.0±0.0	7.5±1.0	10.3±2.8	12.6±4.5	1.3±0.2	1.6±0.3	1.9±0.8	0.0	26.7	40.0	0.0	6.7	20.0
4	0.10	0.05	7	21.4	23.6	28.6	1.0	1.0	3.4	8.4	13.4	17.8	2.4	3.2	5.0	20.0	100.0	100.0	0.0	0.0	0.0
4	0.10	0.05	8	18.8	19.8	24.8	1.0	1.0	1.0	5.2	7.0	9.0	1.0	1.0	1.0	0.0	0.0	80.0	0.0	0.0	0.0
4	0.10	0.05	9	17.4	19.4	27.6	1.0	1.0	3.0	9.8	13.4	17.6	1.8	3.2	3.6	20.0	100.0	100.0	0.0	0.0	0.0
			mean	19.2±2.0	20.9±2.3	27.0±2.0	1.0±0.0	1.0±0.0	2.5±1.3	7.8±2.4	11.3±3.7	14.8±5.0	1.7±0.7	2.5±1.3	3.2±2.0	13.3	66.7	93.3	0.0	0.0	0.0
5	0.10	0.10	7	18.2	23.4	46.0	1.0	1.2	2.8	9.0	12.2	17.4	2.0	3.2	4.4	20.0	100.0	100.0	0.0	0.0	0.0
5	0.10	0.10	8	18.8	20.0	27.0	1.0	1.0	1.0	5.6	8.8	11.8	0.8	1.0	1.6	0.0	40.0	100.0	0.0	0.0	0.0
5	0.10	0.10	9	18.8	21.4	30.4	1.0	1.0	1.0	5.6	9.8	14.2	1.0	1.2	2.2	0.0	100.0	100.0	0.0	0.0	0.0
			mean	18.6±0.3	21.6±1.7	34.5±10.1	1.0±0.0	1.1±0.1	1.6±1.0	6.7±2.0	10.3±1.7	14.5±2.8	1.3±0.6	1.8±1.2	2.7±1.5	6.7	80.0	100.0	0.0	0.0	0.0
6	0.20	0.10	7	34.8	39.0	41.6	1.0	1.0	3.0	11.6	14.2	17.2	3.6	4.2	4.2	100.0	100.0	100.0	0.0	0.0	0.0
6	0.20	0.10	8	20.4	21.6	23.2	1.6	1.8	1.8	7.8	8.8	12.0	2.0	2.0	2.2	40.0	60.0	100.0	0.0	0.0	0.0
6	0.20	0.10	9	23.1	23.5	28.1	1.2	1.3	2.3	11.9	12.9	15.7	3.3	4.0	4.1	100.0	100.0	100.0	0.0	0.0	0.0
			mean	26.6±7.4	28.4±9.3	31.8±9.3	1.2±0.3	1.3±0.5	2.4±0.6	11.1±3.1	12.7±3.4	15.6±3.1	3.2±1.1	3.7±1.6	3.8±1.4	80.0	86.7	100.0	0.0	0.0	0.0

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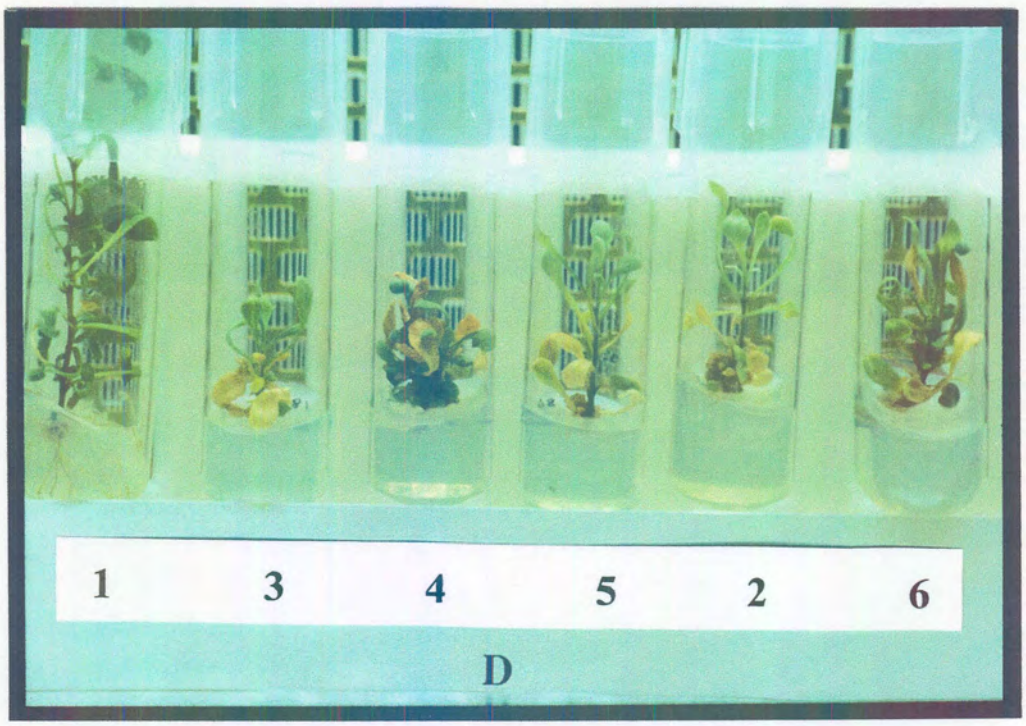

Figure 2 Plant number nine of trial three of *A. nummularia* at the six different treatments. The treatments were: 1: 0.00mg.l^{-1} BA and 0.00mg.l^{-1} NAA, 2: 0.20mg.l^{-1} BA and 0.05 mg.l^{-1} NAA, 3: 0.00mg.l^{-1} BA and 0.05 mg.l^{-1} NAA, 4: 0.10mg.l^{-1} BA and 0.05 mg.l^{-1} NAA, 5: 0.10mg.l^{-1} BA and 0.10mg.l^{-1} NAA and 6: 0.20mg.l^{-1} BA and 0.10 mg.l^{-1} NAA.

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Trial 4

After four weeks the material from Trial 3 was divided and transferred to fresh media with the same six treatments. This was Trial 4. Measurements were taken over a period of only two weeks. This was not comparable with the other trials but there was unfortunately a time limitation on this trial. Enough material of plant number one was available and this plant was included. The results are presented in Table 15.

The statistical analyses were done for plant height, shoot number, number of buds and number of leaves. No significant ($p<0.025$) differences were found between treatments, but there were significant differences between plants.

The average height of plant seven was significantly ($p<0.025$) higher than that of plant eight for all treatments in week two. The average leaf number of plant seven was significantly ($p<0.025$) higher than that of plant eight for week one and than that of plants one and eight for week two for all six treatments. Plant seven had significantly more buds than plant one for all the treatments in week two. These results indicates that plant seven is better adapted to micropropagation than either plants one or eight with plant nine being intermediate. These results might be due to inherent genetic differences between different plants from the same species. It is, therefore, important to use a mixture of many different plants from the same species when research is done to find an *in vitro* propagation procedure for a new plant species.

Treatment one had the tallest shoots with an average shoot height of 24.3 mm and, together with treatment two, was the only treatments where root formation occurred. Treatment one might thus still be the best treatment for elongation and rooting in comparison with the other five treatments. The other measurements were, on average, very much the same, with treatment two producing slightly more shoots and leaves than the other treatments and virtually the same number of buds as treatments three and six.

Treatment two was once again the best medium for multiplication. Treatment one, the basal medium without any plant growth regulators, was throughout this experiment, the best medium for rooting.

Yan-Xiu, Dun-Yi & Harris (1993) tested different concentration of the auxin IBA (indole buteric acid) for the *in vitro* rooting of *Sesbania cannabina*, *S. bisponosa*, *S. formosa* and *S. sesban*, and also found that the MS basal medium, without any growth regulators, induced the best rooting.

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Table 15 Average values of the measured parameters of Trial 3 for *A. nummularia* at week 1 and week 2 after culture initiation. Ten cultures were evaluated per treatment per plant.

Treatment	BA	NAA	Plant	Height (mm)		Shoots (no.)		Leaves (no.)		Buds (no.)		Callus (%)		Roots (%)	
no.	mg.l^{-1}	mg.l^{-1}	no.	week1	week2	week1	week2	week1	week2	week1	week2	week1	week2	week1	week2
1	0.00	0.00	1	23.0	36.8	1.0	1.6	7.6	12.3	1.1	0.9	0.0	0.0	0.0	30.0
1	0.00	0.00	7	17.4	23.7	1.0	1.1	8.4	16.3	1.4	2.2	0.0	50.0	0.0	0.0
1	0.00	0.00	8	17.1	19.3	1.0	1.2	4.8	9.6	0.8	1.2	0.0	0.0	0.0	0.0
1	0.00	0.00	9	14.3	17.3	1.0	1.2	6.2	11.3	1.1	3.0	0.0	33.0	0.0	20.0
		mean		18.0±3.6	24.3±8.8	1.0±0.0	1.3±0.2	6.8±1.6	12.4±2.8	1.1±0.2	1.8±1.0	0.0	20.8	0.0	12.5
2	0.20	0.05	1	15.0	21.0	1.0	1.7	3.7	8.0	0.7	1.0	0.0	33.0	0.0	0.0
2	0.20	0.05	7	20.1	25.1	1.0	1.5	11.1	20.0	1.6	5.0	0.0	63.0	0.0	0.0
2	0.20	0.05	8	13.9	19.4	1.2	1.9	6.2	9.3	1.5	1.8	0.0	20.0	0.0	0.0
2	0.20	0.05	9	13.7	18.5	1.4	2.4	10.6	16.5	3.4	4.8	0.0	80.0	0.0	0.0
		mean		15.7±3.0	21.0±2.9	1.2±0.2	1.9±0.4	7.9±3.6	13.5±5.7	1.8±1.1	3.2±2.0	0.0	49.0	0.0	0.0
3	0.00	0.05	1	19.3	21.6	1.0	1.0	7.3	12.0	1.8	3.1	0.0	0.0	0.0	25.0
3	0.00	0.05	7	17.2	24.5	1.0	1.4	7.9	13.2	1.0	1.9	0.0	50.0	0.0	40.0
3	0.00	0.05	8	13.0	14.0	1.0	1.5	6.3	10.9	2.0	2.4	0.0	0.0	0.0	0.0
3	0.00	0.05	9	11.8	12.2	1.0	1.0	6.3	9.0	1.0	1.3	0.0	17.0	0.0	0.0
		mean		15.3±3.5	18.1±5.9	1.0±0.0	1.2±0.3	6.9±0.8	11.3±1.8	1.4±0.5	2.2±0.8	0.0	16.8	0.0	16.3
4	0.10	0.05	1	11.8	16.3	1.0	1.2	6.5	11.7	0.8	2.3	0.0	0.0	0.0	0.0
4	0.10	0.05	7	13.9	24.9	1.0	2.0	11.1	17.4	1.1	6.4	0.0	80.0	0.0	0.0
4	0.10	0.05	8	14.3	16.0	1.0	1.0	4.1	7.0	0.8	1.5	0.0	25.0	0.0	0.0
4	0.10	0.05	9	17.5	20.6	1.0	1.6	7.0	15.0	0.6	3.6	0.0	50.0	0.0	0.0
		mean		14.4±2.3	19.5±4.2	1.0±0.0	1.4±0.5	7.2±2.9	12.8±4.5	0.8±0.2	3.5±2.1	0.0	38.8	0.0	0.0
5	0.10	0.10	1	15.3	22.5	1.0	1.3	5.3	10.0	1.0	1.3	0.0	67.0	0.0	0.0
5	0.10	0.10	7	18.0	24.8	1.0	1.2	7.7	17.3	0.7	3.9	0.0	70.0	0.0	0.0
5	0.10	0.10	8	20.1	21.9	1.0	1.3	4.9	8.2	1.1	1.8	0.0	10.0	0.0	0.0
5	0.10	0.10	9	13.8	17.0	1.0	1.4	7.2	11.8	1.3	3.1	0.0	78.0	0.0	0.0
		mean		16.8±2.8	21.6±3.3	1.0±0.0	1.3±0.1	6.3 ±1.4	11.8±3.9	1.0±0.3	2.5±1.3	0.0	56.3	0.0	0.0
6	0.20	0.10	1	17.0	22.8	1.0	1.2	5.0	8.8	0.2	1.0	0.0	50.0	0.0	0.0
6	0.20	0.10	7	12.3	17.9	1.0	1.8	7.7	17.0	1.1	5.0	0.0	100.0	0.0	0.0
6	0.20	0.10	8	14.5	16.0	1.0	1.0	6.0	11.0	1.0	3.0	0.0	100.0	0.0	0.0
6	0.20	0.10	9	11.3	21.0	1.0	1.7	8.3	14.7	1.7	5.0	0.0	67.0	0.0	0.0
		mean		13.8±2.5	19.4±3.1	1.0±0.0	1.4±0.4	6.8±1.5	12.9±3.7	1.0±0.6	3.5±1.9	0.0	79.3	0.0	0.0

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On the other hand Datta, Datta & Tramanik (1983) experienced high rooting percentages for another woody species, *Dalbergia sissoo*, when applying various combinations of IAA, NAA and Kn (kinetin) to the MS basal medium. For the multiplication and elongation it is suggested that other auxins, cytokinin and also gibberellic acid also be tested, as Wochok & Sluis (1980) and Tripathy & Goodin (1989) had similar and better results with *A. canescens* using other plant growth regulators than BA and NAA, which were used in this experiment for *A. nummularia*.

The results from this experiment were, however, not yet good enough to be described as the best media for the *in vitro* propagation of *A. nummularia*. After these trials it can be said that it is possible to multiply elite *A. nummularia* plants *in vitro*, but the technique still needs considerable refinement to optimize it and to make it more economical.

Acclimatization

The results of the acclimatization phase are illustrated in Table 16. From Table 16 it can be seen that all ten plants grew slowly for the first two months of transplanting. The reason was that the plants with a spindly growth form were pruned, to stimulate shoot multiplication and to obtain a multi-stemmed plant, as such a growth form will be more suitable for grazing; more shoot tips will be produced to use for cuttings or initiation of plant tissue cultures; more shoots will result in higher seed yields, which would be needed for future trials with this elite material. This is the reason why most of the plants had a lower height at two months (average 4.4 cm.) than at one month (average 5.0 cm) after planting.

Table 16 Average plant heights (cm) of the 10 plants at monthly intervals, after transplanting.

Number of plants	Plant nr.	Months after transplanting			
		1	2	3	4
10	1	5.8	4.4	5.7	14.0
11	2	7.1	5.3	7.4	14.0
4	3	5.1	5.6	7.4	19.4
11	4	7.9	4.8	8.3	19.6
7	5	5.7	5.4	8.2	18.8
5	6	7.6	4.9	7.8	19.2
2	7	1.8	2.4	6.5	19.8
2	8	2.3	3.3	9.3	21.0
9	9	1.7	2.8	7.3	22.9
12	10	5.0	5.5	11.0	14.7
	average	5.0	4.4	7.9	18.3

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Another reason, with the exception of the plants still acclimatizing morphologically, why the plants did not grow well during the first two months might have been the season, as it was late autumn, May and June in Pretoria, with relatively low temperatures and a shorter photo period, while the plants were used to high temperatures and a longer photo period. After two months growth rates increased and by the fourth month most of the plants had reached heights of approximately 20 cm. Plant numbers one and two did not grow as well as the other plants, but their growth was acceptable.

In Figure 3 the percentage survival of the ten plants at an age of four months after transplanting is illustrated. Except for plant number nine, with a survival rate of 56%, all the plants showed survival rates of between 73% and 100%. The average survival rate of 89% is acceptable and indicates that the method that was tested for acclimatization was satisfactory. According to Goussard & Wiid (1989) the acclimatization of *in vitro* cultured vinelets by means of immersion of root systems in standard nutrient solutions, holds numerous advantages. Their method comprised the immersion of the root systems of ex *in vitro* grapevine cultures in inorganic nutrient solutions. Rapid growth proceeded under controlled conditions.

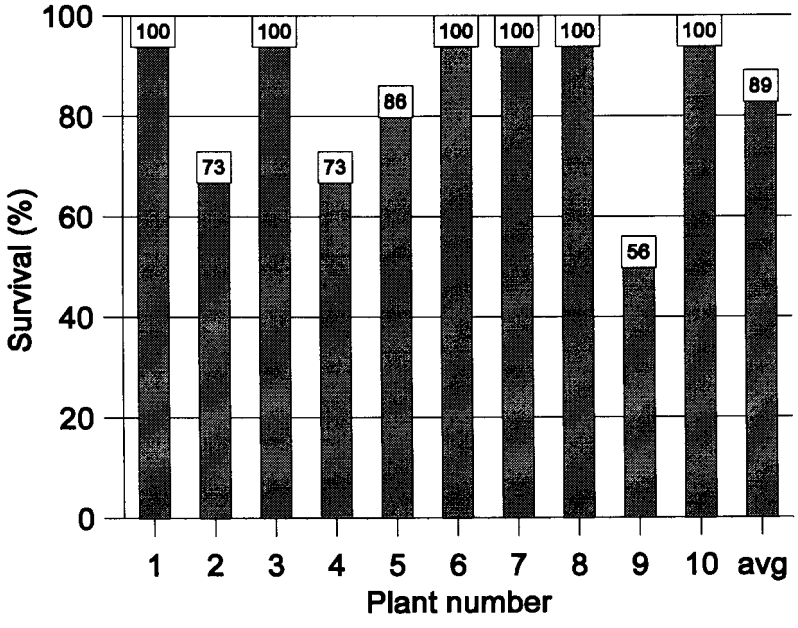

Figure 3 Percentage survival of *Atriplex nummularia* four months after transplanting from *in vitro* to *ex vitro* conditions

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Following the emergence of well-developed leaves above the top of the tubes, the vinelets were transplanted to soil media without any covering with glass or plastic tents. Percentage survival varied from 95% to 100%. This method could also be tested for *Atriplex* species in the future if the need should rise for a method with an even higher success rate than the one tested for this trial.

CONCLUSIONS AND RECOMMENDATIONS

Crop improvement usually relates to yield and\or the economics of production. The potential of micropropagation has not yet been fully realized, partly because of the high labour cost involved and partly because of the lack of commercial return allowing for the investment in automation technology. The push toward healthy diets and good nutrition will continue and probably increase. This means that fruit and vegetables, low in cholesterol and fat, will continue to be in high demand (Bhojwani, 1990). This statement might be a threat for the future of meat production. If, on the other hand, one takes the strong influence of culture and background on diet composition of man into account, there might still be a future for meat production. This will largely be in third world countries where the subsistence farmer can or will, not invest in costly seedlings. What about labour in these countries? There will never be any clarity about the future of *in vitro* propagated fodder shrubs in especially third world countries, until it is intensively investigated. Labour is not that expensive in third world countries. This opens the possibility of producing *in vitro* plant material, which is usually very labour intensive, at relatively low rates.

Propagation of elite *Atriplex* species through tissue culture is possible. It is a quick method of producing many homogenous plants in a very short time and even out of season. The only disadvantage is the expense. Micropropagation is a costly method for propagating an extensive species like *Atriplex*, but, as stressed by Bhojwani (1990), it is a quick method for plant production and preservation. In this case, promising individuals can be preserved for future studies. Another risk is that the end result may be many clones of the same plant, which means that the entire population might be destroyed if there is any one disease the mother plant is not resistant to. Up to date no such diseases or pest have been reported in literature on *Atriplex* under South African conditions.

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The first of two main advantages of propagating *Atriplex* using tissue culture techniques is that selected material can easily be transferred from one country to another, as sterile cultures. The other reason, as also stressed by Suttle (1992), would be to increase the number of the newly selected material quickly and at some point continue with more traditional methods of propagation such as cuttings. According to Gamborg & Phillips (1995) the possibility exists of producing as many as 1 000 000 plants from a single plantlet within a period of six months. Using axillary buds, as was partly done in this study, produces the smallest number of plantlets, as the number of shoots produced is limited by the number of axillary buds transferred to each test tube (Bhojwani, 1990). Adventitious bud formation, buds developing from other tissues like stem-, root- or leaf tissues, has a greater potential for multiplication, as shoots may develop from any part of the inoculum. Somatic embryo genesis has the potential for producing the greatest number of plantlets, as it is the initiation and development of embryos from non-sexual cells. Unfortunately this method can only be induced in a limited number of species (Bhojwani, 1990 and Gamborg & Phillips, 1995). In this trial somatic embryo genesis was not tested. It is suggested that somatic embryo genesis be tested for *Atriplex* species to enhance the *in vitro* multiplication rates of *Atriplex* species.

During this study an easy method for disinfestation of *A. nummularia* was found which might also be suitable for the disinfestation of *A. canescens*. It is, however, important to first produce greenhouse grown mother plants by making stem cuttings after the selection of elite material in the field. This will limit contamination during the disinfestation stage. Media, potentially suitable for multiplication, elongation and rooting, were found for *A. nummularia* and *A. canescens* but refinements of these media to improve results are suggested by testing a wider range of auxins and cytokinins at different concentrations and combinations. To enhance shoot elongation it is suggested that gibberellic acid should also be tested as Wochok & Sluis (1983) found good response of *A. canescens* to this treatment. A further suggestion is that only ½-strength MS basal medium should also be tested. There are four reasons: (1) Tripathy & Goodin (1989) found better culture development for *A. canescens* on ½-strength MS basal medium than the full strength medium; (2) according to Ziv (1991) it might lower the appearance of vitrification of which signs were noticed in this trial; (3) woody species like *Eucalyptus radiata* grew better on ½-strength MS medium (Donald & Newton, 1991) and *Atriplex* is a semi-woody species; and (4) it will be cost effective as only half the amount of chemicals will be used to prepare the media.

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Acclimatization of rooted *A. nummularia* cultures was possible with great success, while the rooting of *A. canescens* cultures needs more attention before its acclimatization can be tested.

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CHAPTER 6

VEGETATIVE PROPAGATION OF *ATRIPLEX NUMMULARIA* L.

ABSTRACT

Atriplex nummularia (L.) is a well known fodder shrub in the arid and semi-arid parts of South Africa. Differences in palatability within the species necessitated the propagation of more palatable plants. As *Atriplex* is open pollinated, vegetative propagation was tested. It was decided to use stem cuttings for this purpose. The trial was conducted in a mist-bed and replicated over three seasons. A pilot trial was first conducted where terminal cuttings of 10 palatable *A. nummularia* plants were rooted. The results ranged from 53% to 100% rooting, which indicated that the proposed method could be tested further. Two plants were used: one with new growth which had been pruned to 45 cm. the previous season and one with old growth which had not been pruned the previous season. For each plant two cuttings were tested: terminal and subterminal. Each type of cutting was treated with three hormonal regimes: 0 g.kg-^{1}, 3 g.kg-^{1} and 8 g.kg-^{1} IBA (indole-3 butyric acid). For each hormonal regime 10 cuttings were used. All treatments were replicated three times. The best results were obtained in spring. In both plants the performance of the sub-terminal cuttings was poor. For the plant with old growth, the terminal cuttings had acceptable root initiation, but not as good as the terminal cuttings of the new growth plant. With respect to the hormonal treatments, the 0 g.kg^{-1} IBA showed an average (over all treatments) root initiation of 36%, while the 8 g.kg^{-1} IBA showed an average root initiation of 46%. The best overall results were from the terminal cuttings of the new growth plant, treated with 3 g.kg^{-1} in spring which had 85% rooting. It is, therefore, clear that it is possible to propagate *A. nummularia* vegetatively, using terminal cuttings of the new growth in spring and treated with 3 g.kg^{-1} IBA. With further refinements, large scale vegetative propagation of palatable *A. nummularia* plants through cuttings, should be feasible.

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INTRODUCTION

Atriplex nummularia (old man saltbush) is a naturalised and well known fodder shrub in the arid and semi-arid areas of South Africa. Stock farmers establish plantations of *A. nummularia*, to be used as a fodderbank during dry periods, when the carrying capacity of natural grazing is very low (Barnard, 1986). Differences in palatability within the species necessitate the propagation of more palatable plants. A more homogenous stand of palatable plants, should ensure a more effective utilization of *A. nummularia* plantations. As the genus *Atriplex* is dioecious and open pollinated (Osmond, Björkman & Anderson, 1980), vegetative propagation was evaluated. It was decided to use stem cuttings for vegetative propagation.

According to the literature similar trials have been conducted on other *Atriplex* species such as *A. amnicola, A. canescens*, *A. cuneata*, *A. confertifolia*, *A. halimus* and *A. lentiformis* (Ellern, 1971; Wiesner & Johnson, 1977; Everett, Meeuwig & Robbertson, 1978; Richardson, Barker, Crofts & Van Epps, 1979; Sen & Rajput, 1995). De Kock (1983) mentioned that it is possible to root stem cuttings of *A. nummularia*, but did not quantify his success rate.

According to Larsen & Guse (1975) and Panetsos (1993) rooting success is dependent upon many endogenous and exogenous variables. The endogenous factors include maturation of the cutting donor, type of cutting, physiological condition of the cuttings, preconditioning of the cuttings or cutting donor and season of collection. The exogenous factors include rooting medium, ambient temperature and humidity, photo period, light intensity, hormone and chemical treatments. The degree of control required for each of these variables is directly dependent upon the rooting ability of the species in question.

In order to determine the most effective way of propagating *A. nummularia* vegetatively, most of these above mentioned variables were taken into account during this trial. In this case it can be assumed that the *Atriplex* species is an easy rooter, as some evidence does exist in the literature (Ellern, 1971; De Kock, 1983). Sen & Rajput (1995) stressed that vegetative propagation is the most suitable method for propagating *A. amnicola*. *Atriplex amnicola* possesses the ability to form nodal roots during the rainy season which helps in the production of a large number of rooted cuttings for field planting.

The specific research objectives were to: identify the optimum season to collect stem cuttings of *A. nummularia*; determine if the age of the cutting played an important role; determine how the portion of stem used for cuttings affected rooting and to study the response of *A. nummularia*

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to various concentrations of exogenously applied indole-3-butyric acid (IBA).

MATERIAL AND METHODS

Pilot trial

The trial was conducted on the Hatfield Experimental Farm of the University of Pretoria. It was started with a pilot trial to determine whether the proposed procedure would be effective. For this trial 10 different *A. nummularia* plants were selected on basis of palatability and persistence under grazing. Only the terminal sections of young branches were used. The cuttings were placed in plastic bags, containing wet paper to keep them moist, immediately after they were harvested and then transported to the mist bed. Cuttings were approximately 15 centimetres (cm) long, with the lower 10 cm leaves removed. The lower 3 cm. of each cutting was wounded by removing some of the bark, to ensure better contact between the plant tissue and the IBA (Everett *et al*, 1978). This portion of the cutting was dipped first in water and tapped against the side of the container to remove excess water. The moistened cuttings were then dipped in commercial talc powder, containing 3 $g.kg^{-1}$ IBA, and excess powder was removed by tapping the cuttings against the inside of the container. IBA talc powder was used because IBA has been shown to be probably the best hormone for general use due to its effectiveness in promoting root initiation of a large number of plant species and its lack of general toxicity over a wide concentration range (Hartman, Kester & Davies, 1990). Cuttings were then placed to depth of 5 cm. in a mist bed containing sand. The mist bench sprayed mist for 8 seconds, every 4 minutes, to maintain a high humidity. The temperature of the mist bed was set to approximately 30°C. Cuttings were removed from the mist bed when they had rooted or degenerated. This was four to five weeks after initiation. Five of the rooted cuttings, of each of the 10 individual plants, were planted in four litre (l) pots containing 40% soil, 40% compost and 20% sand. The pots were placed on a hardening off table for three days and sprayed with water every 15 minutes for eight seconds for the first day and every 30 minutes for the rest of the period. From there the pots were transferred to a greenhouse.

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Main trial

The cuttings were taken from an existing *A. nummularia* plantation. Two different plants: one with new growth which had been pruned to 45 cm. the previous season and one with old growth as it had not been pruned the previous season, were used. This was done to determine if the age of the material would influence rooting ability. The cuttings were placed in plastic bags, containing wet paper to keep them moist, immediately after they were harvested and then transported to the mist bed.

For each plant two types of cutting were tested: terminal and subterminal. The terminal cuttings from the new growth plant were juvenile growth and more herbaceous, while the other cuttings were more semi-hardwood than herbaceous.

Each type of cutting was treated with three hormonal regimes: 0 $g.kg^{-1}$, 3 $g.kg^{-1}$ and 8 $g.kg^{-1}$ IBA. For each plant, cutting type and hormonal regime, 10 cuttings were used. The same procedure was used as described for the pilot trial. All the treatments were executed in four replicates and replicated over three seasons: summer, winter and spring. For each season 480 cuttings were taken, 240 for each plant and 60 for each replicate, with each replicate consisting of 30 terminal and 30 subterminal cuttings, 10 for each hormonal treatment.

Statistical analyses, where applicable, were conducted with the ANOVA procedure to determine significant differences ($P < 0.05$) and a Response Surface analysis were also done. For this the SAS (SAS, 1994) programme was used.

RESULTS AND DISCUSSION

Pilot trial

The results of the pilot trial are illustrated in Table 1. Large differences in the percentage rooting between individual plants were visible. For example, between plant number (nr.) 7 with 100% rooting and plant nr. 9 with only 53% rooting. This might be a result of the genetic variation within *A. nummularia*, which might also be the cause of the difference in palatability.

Both Weisner & Johnson (1977) and Richardson *et al* (1979) were concerned about the fact that the genus *Atriplex* is dioecious and that male and female plants might have different rooting

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abilities. They found that the sex of plants had no influence on the rooting ability of either *A. canescens* or *A. confertifolia*. In this trial the sexes of the 10 different plants used, are illustrated in Table 1. It was also found that plant sex had no influence on the rooting ability of *A. nummularia*, as plants nr.1(♀) and 2(♂) showed the same rooting ability and plants nr. 3(♂) and 6(♀) also showed the same rooting ability.

Table 1: Percentage rooting of 10 individual *A. nummularia* plants

Plant nr.	Number of cuttings	Number of rooted cuttings	Rooted cuttings %
1(♀)	20	17	85
2(♂)	12	10	83
3(♂)	20	11	55
4(♀)	20	16	80
5(♂)	10	7	70
6(♀)	15	8	53
7(♀)	20	20	100
8(♂)	20	17	85
9(♀)	15	8	53
10(♀)	15	12	80

Of the 50 cuttings (5 from each of the 10 individual plants) that were transplanted for hardening off, 46 survived and grew into mature plants. This was a survival rate of 92%, and seems to be acceptable (Weisner & Johnson, 1977).

It was clear that rooting of *A. nummularia* cuttings, following the proposed procedure, was possible and it was decided to continue with the main trial to quantify the procedure.

Main trial

The results of the main trial are illustrated in Table 2. The three seasons were, summer, autumn and spring and the two plants that were used were one with old growth and one with new growth. The results will be described in more detail for season, plant type, cutting type and IBA treatment.

The results illustrated here are only the average over all replications. The best combination of season, plant type, cutting type and IBA treatment will be discussed separately.

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Table 2: Average % rooting of *A. nummularia* cuttings. Where T1 represents 0 g.kg-^{1}, T2 represents 3 g.kg-^{1} and T3 represents 8 g.kg-^{1} IBA.

Season	Old growth plant								New growth plant								Total mean
	Terminal cuttings				Subterminal cuttings				Terminal cuttings				Subterminal cuttings				
	T1	T2	T3	mean	T1	T2	T3	mean	T1	T2	T3	mean	T1	T2	T3	mean	
Summer	35 ± 13	60 ± 8	68 ± 10	54 ± 17	30 ± 20	15 ± 6	28 ± 10	24 ± 14	28 ± 15	30 ± 42	55 ± 24	38 ± 30	13 ± 19	43 ± 31	45 ± 24	34 ± 27	37 ± 25
Mean (plant type)					39 ± 22							35 ± 28					
Autumn	45 ± 13	55 ± 24	43 ± 26	48 ± 21	0 ± 0	0 ± 0	3 ± 5	1 ± 3	88 ± 5	90 ± 8	95 ± 10	91 ± 8	23 ± 5	38 ± 28	28 ± 25	30 ± 20	42 ± 36
Mean (plant type)					24 ± 28							60 ± 35					
Spring	73 ± 17	75 ± 6	65 ± 17	71 ± 14	18 ± 13	15 ± 17	3 ± 5	12 ± 13	68 ± 21	88 ± 19	85 ± 6	80 ± 18	18 ± 15	38 ± 34	18 ± 5	25 ± 21	47 ± 34
Mean (plant type)					41 ± 33							52 ± 34					
Mean (cutting type)			Terminal cutting: 63 ± 26						Subterminal cutting: 21 ± 21								
Mean (T)	51 ± 21	63 ± 16	59 ± 20	58 ± 20	16 ± 18	10 ± 12	11 ± 14	12 ± 15	61 ± 29	69 ± 38	78 ± 22	70 ± 30	18 ± 14	40 ± 28	30 ± 22	29 ± 23	
Total mean					35 ± 29							50 ± 34					

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Season

Season had a significant ($p<0.05$) effect on the percentage rooting. Percentage rooting of cuttings were higher in the spring (47%) than in the summer (37%), with the autumn (42%) intermediate. During the summer, the high temperatures in the greenhouse, together with the high humidity, created excellent conditions for disease development in the mist bed, which consequently contaminated most of the cuttings. This probably contributed to the low rooting percentage during the summer. The best results, on average for all treatments, were obtained in spring. Although 47% rooting appears to be low, it is the average over all treatments. Within the treatments rooting successes of up to 85% were achieved (see Table 2) in spring. Figure 1 illustrates the difference in IBA treatments in the three seasons, for terminal cuttings. From this figure it is clear that, for all three IBA treatments, rooting was higher in spring than in summer or autumn and higher in autumn than in summer. Although the overall rooting in autumn is not significantly lower than in spring, rooting of the old growth plant material was lower than in any other season (Table 2). This might be an effect of more mature material, giving lower rooting, or a endogenous genetic variation between the two plants used. It would, therefore, be recommended that spring is a better season than autumn to propagate *A. nummularia* vegetatively.

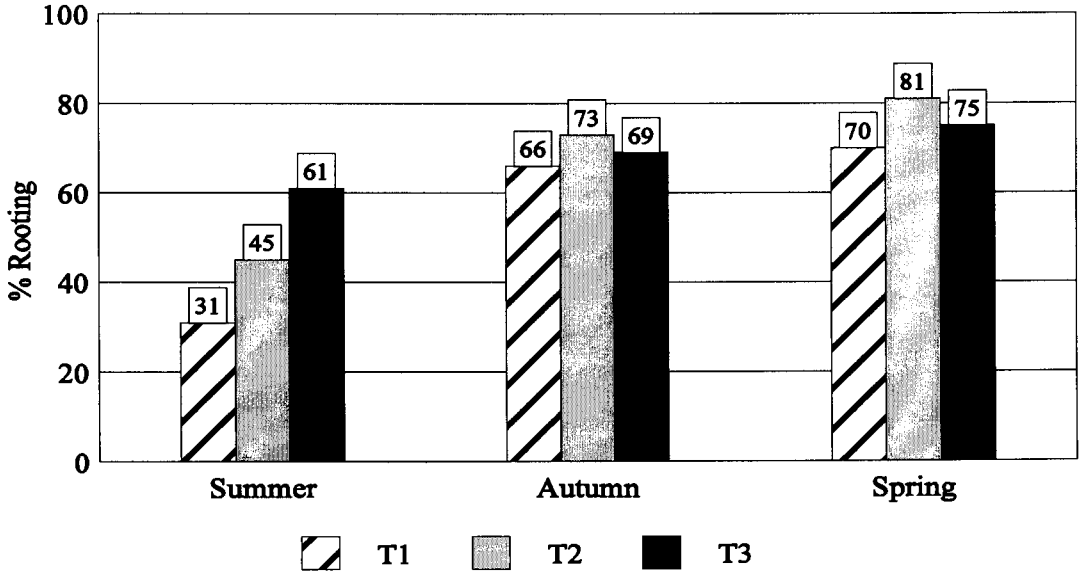

Figure 1 Average rooting of terminal cuttings of both plant types over three seasons. Where T1 represents 0 g.kg-^{1} , T2 represents 3 g.kg-^{1} and T3 represents 8 g.kg-^{1} IBA.

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Type of plant

Both the type of plant and the interaction between season and type of plant had a highly significant ($p<0.01$) effect on the percentage rooting. Rooting of the new growth plant material was, for most of the treatments higher than that of the old growth plant material. This is also evident from the total mean values of 35% rooting for the old growth plant and 50% for the new growth plant. These results are similar to those of Arya, Toky, Tomar, Singh & Harris (1993) whose study indicated that stem cuttings of juvenile material gave the best results with *Prosopis cineraria*(L.). However, Ellern (1971) could find no differences between the rooting ability of "soft" and "woody" cuttings of *A. halimus*.

For the old growth plant material, the terminal cuttings had acceptable rooting (58%), but not as good as the terminal cuttings of the new growth plant material (70%). According to Figure 2 the largest difference between the two plant types was in autumn. During autumn and winter the plants are relatively dormant, which might explain the poor (24%) rooting of the old growth plant material in Figure 2.

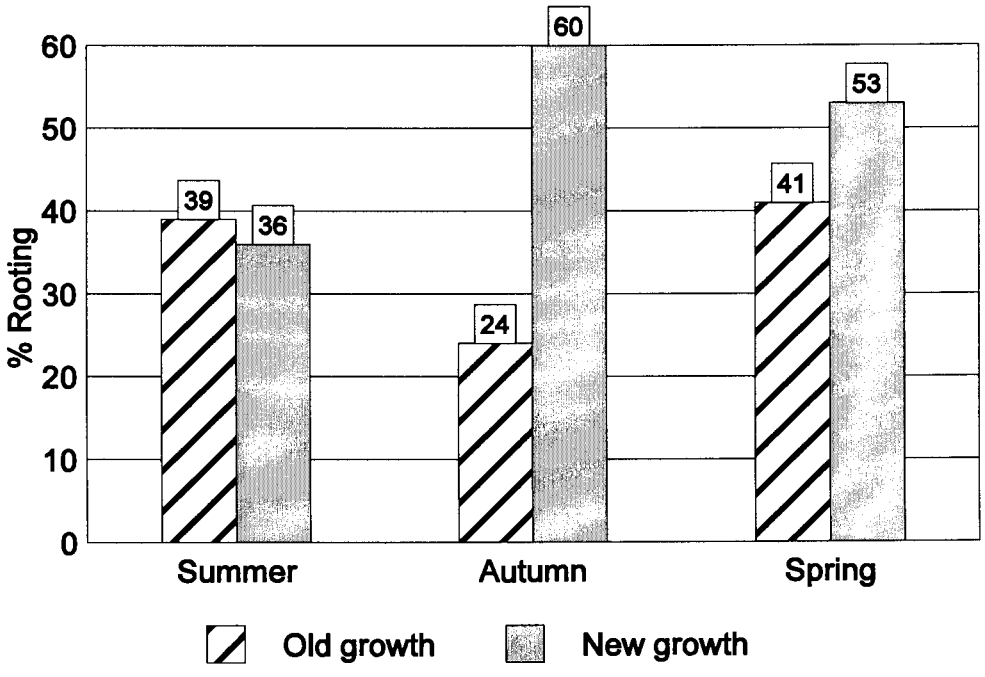

Figure 2 Average percentage rooting of both types of cutting from the old growth and new growth plants over three seasons.

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Richardson *et al* (1979) found the lowest rooting percentages for *A. canescens* in winter. They used only old growth plants. The high rooting percentage of the new growth plant material in autumn, (see Figure 2) is difficult to explain. It might be that the new growth reached dormancy at a later stage than the old growth, or that new growth had higher levels of carbohydrates, nitrogenous compounds and auxins than old growth, as there is a strong relation between these compounds and root formation (Joshi, Sharma, Shamet & Dhiman, 1992).

Type of cutting

Both type of cutting and the interaction between season and type of cutting had a highly significant ($p<0.01$) effect on percentage rooting. In both plants the performance of the subterminal cuttings was relatively poor. In all three seasons rooting of terminal cuttings was higher than rooting of subterminal cuttings. The mean values are illustrated in Figure 3. Although the largest percentage of rooting for terminal cuttings was in autumn (Table 2), the best overall average was in spring as can be seen in Figure 3.

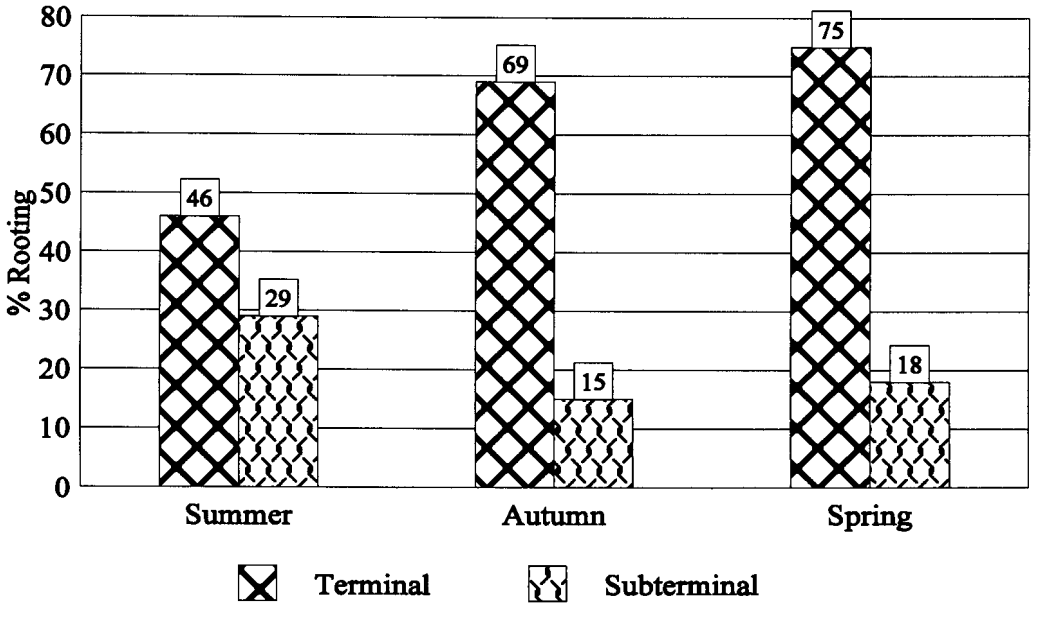

Figure 3 Average percentage rooting of terminal and subterminal cuttings over three seasons for both plant types.

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According to Hartman *et al* (1990), the type of cutting can vary between species. For easily rooted species, like *A. amnicola* (Sen & Rajput, 1995), type of cutting is of little or no importance. This was also true for Everett *et al* (1978) who found no difference in rooting ability between different types of cuttings of *A. canescens* and *A. confertifolia*.

Hormonal treatment

Hormonal treatment had a significant ($p<0.05$) effect on the percentage rooting. The 0 $g.kg^{-1}$ IBA had an average root initiation of 36%, while the 3- and 8 $g.kg^{-1}$ IBA had an average root initiation of 46% and 45% respectively. When only the terminal cuttings were evaluated (Figure 1), 3 $g.kg^{-1}$ IBA gave the best results in autumn (73%) and spring (81%), with the average of 81% in spring being higher than any other hormonal treatment. The interaction between treatment and season also had a significant ($p < 0.05$) effect on the percentage rooting. The 0 $g.kg^{-1}$ IBA treatment had a 70% rooting success in the spring for terminal cuttings, which is promising in the sense that it would be much more economical if *A. nummularia* could be propagated vegetatively without auxin treatment. It might even be possible to find some *A. nummularia* plants with the genetic potential to have a rooting percentage of above 70%. Ellern (1971), for example, recorded 77% rooting for *A. halimus*, without any hormonal treatment.

CONCLUSION AND RECOMMENDATIONS

From these results it is clear that vegetative propagation of *A. nummularia*, by stem cuttings, is feasible. According to the Response Surface Analysis (SAS 1994) that was conducted, to determine the optimum method for rooting stem cuttings of *A. nummularia,* the following procedure is recommended. Use a plant with new growth (for instance one that was pruned recently and has already started new shoot growth); use only terminal stem cuttings of 10 to 15 cm; treat the cuttings with 3 $g.kg^{-1}$ IBA and do this in the late autumn or spring. Rooted cuttings can be transplanted into pots containing a mixture of 40% soil, 40% compost and 20% sand. This is, however, only applicable to Pretoria in the Gauteng Province of South Africa. This same method should be tested in other regions, where *A. nummularia* is commonly planted by farmers and, where the provenance grows under different climatic and ecological conditions.

Another recommendation is that the provenance should consist of more than one plant. The one

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reason is the genetic difference between plants within this one species which might result in different rooting abilities between different plants. The other reason is to maintain genetic variability, to protect the progeny against pests. If all the new plants are from the same provenance, they will possibly be highly vulnerable to pests.

Another advantage of vegetatively propagated plants is that they would mature earlier than plants produced by seed. (Hartman *et al*, 1990). According to Everett *et al* (1978) large plants can be obtained after approximately three months, instead of after six months when produced from seed.

One limitation of this experiment was that only two individual plants were used, the old growth and new growth plants. From the pilot trial, where 10 different plants were used it was clear that the genetic difference between individual plants might have lead to the variation in rooting ability between those plants. It would have been better if two or more plants for each of the old growth and new growth types that were tested were used.

With further refinements, large scale vegetative propagation of palatable *A. nummularia* plants through cuttings, should be feasible.

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CHAPTER7

DEVELOPMENT OF A MODEL TO SIMULATE THE DRY MATTER PRODUCTION AND STRUCTURE OF TWELVE *ATRIPLEX* SPECIES

ABSTRACT

It is often necessary to use a non-destructive method for measuring DM production. Shrub height and crown diameter are used as non-destructive indicators of growth. In this trial shrub height, diameter and volume together with clipping of above ground material were used to try and develop a model for estimating DM production of 16 *Atriplex* accessions. The 16 accessions were: *A. amnicola*, *A. breweri*, *A. canescens* (five accessions), *A. cinerea*, *A. glauca*, *A. halimus*, *A. lentiformis*, *A. nummularia*, *A. paludosa*, *A. rhagadioides*, *A. semibaccata* and *A. undulata*. Four models were tested: Model 1, all accessions together in one model for shrub volumes; Model 2, shrub volume for each accession; Model 3 shrub height and diameter for each accession; and Model 4 all accessions together for volume where the slope were the same for all accessions but with different intercepts. The r^2 values for Models 1 and 4 were 0.72 and 0.80 repectively, which were acceptable. For Model 2 r^2 values were acceptable with the exception of *A. semibaccata* (0.28), *A. cinerea* (0.59) and *A. halimus* (0.64). For Model 3 r^2 values were acceptable with the exception of *A. semibaccata* (0.37) and *A. paludosa* (0.66). *Atriplex semibaccata*, with its creeping growth form, was the only accession for which a reliable model could not be found. More accurate models might be developed if shapes other than a cylinder were tested and when more than 12 plants per accession were harvested and measured.

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INTRODUCTION

For research on fodder crops the most common measurement is above ground dry matter (DM) production (Kirkman, Engelbrecht & Cockcroft, 1994). Shrub height and crown diameter are used as non-destructive indicators of growth (Thomson, Mirza & Afzal, 1998). It is often necessary to use a non-destructive method of measuring DM production as this will save time, labour, money and the plant material can be utilized by grazing animals. A technique that is rapid, relatively accurate and requires little training is desirable.

According to Andrew, Noble & Lange (1979) there are three techniques which show promise for estimating shrub forage non-destructively in the field: visual estimation, dimensional measurement and the capacitance probe. Cereti and Rossini (1993) describe a photographic technique to measure shrub biomass indirectly. Maywald, McArthur, Jorgensen, Stevens & Walker (1998) describe a modification of the Adelaide Technique which was used as a non-destructive biomass estimation method for *A. canescens*. This method was used to determine the total biomass production of each plant before a grazing trial began.

Thomson *et al* (1998) showed that aerial biomass can be predicted from shrub height, diameter and volume with reasonable levels of precision ($r^2 > 0.80$) for *Atriplex canescens*.

In this trial dimensional measurements were taken together with clipping of the dry matter and these data were used to try and develop a model for estimating DM production of 16 *Atriplex* accessions.

MATERIAL AND METHODS

The 16 *Atriplex* accessions, as well as the origin of each, are listed in Table 1. The experiment was a randomised block design with four replicates. Each replicate was randomly allocated. Each replicate consisted of the 16 accessions, which were also allocated randomly. For each accession five plants were planted per replicate. The spacing was one metre within each row and three metres between the rows, which gave a density of 3333 plants per hectare (ha). This experiment was used for measuring dry matter production and at the same time dimensional measurements were taken before the plants were clipped.

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Table 1 Names and origin of *Atriplex* accessions evaluated

Accessions	Origin
A. amnicola Paul G. Wilson	Western Australia
A. canescens (Pursh.) (Australia)	Australia
A. canescens (Pursh.) (Rincon)	North America
A. canescens (Pursh.) (Santa Rita)	North America
A. canescens (Pursh.) (Field Reserve 1)	North America
A. canescens (Pursh.) Field Reserve 2)	North America
A. cinerea Poir.	Australia, Europe, South Africa
A. glauca L.	Mediterranean, South Africa
A. halimus L.	Asia, Mediterranean
A. lentiformis ssp. *breweri* (Wats.) Hall&Clem	North America
A. lentiformis (Torr.) Wats. ssp. *lentiformis*	North America
A. nummularia L.	Australia
A. paludosa R. Br.	Australia
A. rhagadioides F. Muell.	Australia
A. semibaccata R. Br.	The Americas, Europe, Australia & South Africa
A. undulata D. Dietr.	South America

The shrub height was measured and two measurements of crown diameter (width) were taken at right angles to each other, within the row and across the row. The average of the two shrub widths was taken as the shrub diameter. From these measurements the shrub volumes were determined as the volume of a cylinder. The formula for the volume of a cylinder is: $v(\text{cylinder}) = \pi r^2 h$, where r = half the diameter (half of the plant width) and h = height (plant height) and $\pi = 3.143$. Shrubs were then cut to a height of 10 cm and separated into leaves, edible twigs and inedible twigs. The different components were then oven dried at 60°C to constant weight. Thereafter the material was weighed to determine the dry matter production of each plant. One plant of each accession was harvested from each of the four replicates at eight, 11 and 15 months after planting. At eight months after planting *A. undulata* was still too small to harvest.

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Four models were tested:

1) All the accessions (188 observations) were used together in one model where shrub volume were related to DM production; [$Y = aX + b$]

2) The shrub volume of each of the 16 accessions (12 observations per accession, except *A. undulata* with only eight observations) were related to its DM production; [$Y = a_{1..16} X + b_{1..16}$]

3) The shrub height and diameter of each of the 16 accessions (12 observations per accessions, except *A. undulata* with only eight observations) were related to its DM production;

[$Y = c_{1..16} X + d_{1..16} Z + b_{1..16}$]

4) All the accessions (188 observations) were used together in one model where shrub volume was related to DM production, but each accession had its own intercept while the slope was the same for all accessions; [$Y = eX + b_{1..16}$].

The regression analyses were done using the SAS statistical programme (SAS/STAT, 1994).

RESULTS AND DISCUSSION

The results are presented by discussing each model separately.

Model 1

For the first model, all the accessions (188 observations) were used together in one model where shrub volume was related to DM production [$Y = aX + b$]. The regression was as follows:

$DM = 553.24X + 129.47$ ($r^2 = 0.72$; $P = 0.0001$)

where,

DM = Dry matter production ($g.plant^{-1}$)

X = Shrub volume (m^3)

The value of $r^2 = 0.72$ indicates that 72% of the variation in DM production could be ascribed to variation in volume. This value is lower than that of Thomson *et al* (1998) who reported levels of precision ranging from 0.80 to 0.90. They, however, worked only on *A. canescens*, while in this trial 16 different *Atriplex* accessions and 12 different species were included. Differences in growth form (dimensional measurements) among the different *Atriplex* accessions most probably contributed to variation in DM per plant resulting in the relatively low level of precision (0.72).

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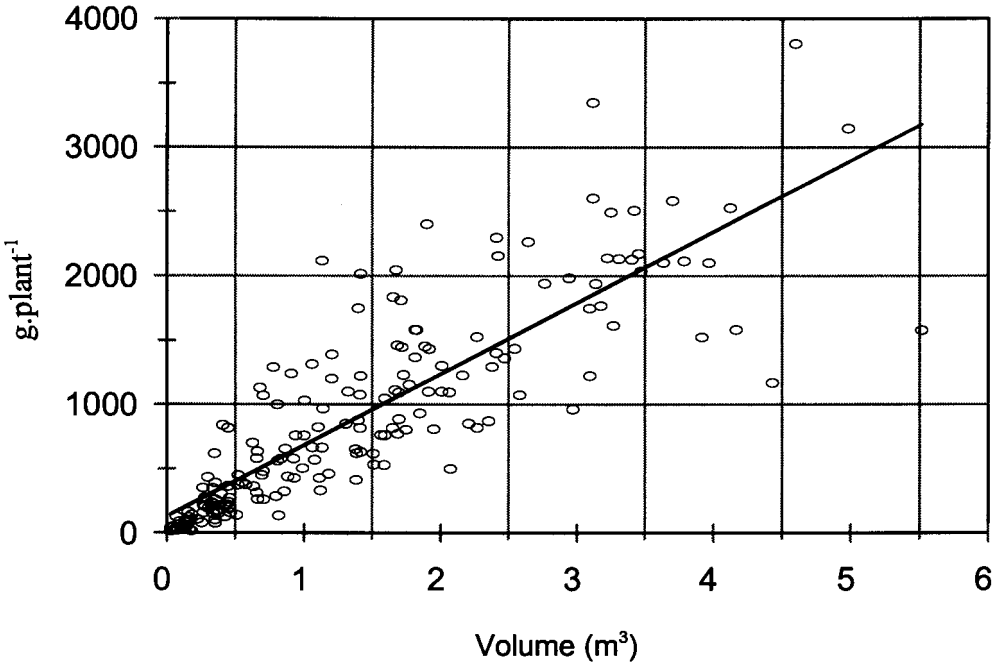

Figure 1 Relationship between total aerial DM production and shrub volume of 16 *Atriplex* accessions

The results for model one are plotted in Figure 1. From this graph it can be seen that more variation occurred where the plants had higher volumes and higher DM production.

Model 2

For the second model the volume of each of the 16 accessions (12 observations per accession, except *A. undulata* with only eight observations) were related to its DM production [$Y = a_{1..16} X + b_{1..16}$]. The regression parameters of the regression analyses are presented in Table 2. Although most r^2 values were relatively high (0.75 - 0.96), those of *A. cinerea*, *A. halimus* and *A. semibaccata* were only 0.59, 0.64 and 0.28 respectively.

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Table 2 Results of the regression between the volume of each accession and its DM production

Accessions	r^2	Intercept (b)	Slope (a)	Significance level
1. *A. amnicola*	0.92	-57.87	711.73	0.0001
2. *A. breweri*	0.86	148.33	566.45	0.0001
3. *A. canescens* (Austr.)	0.93	-30.36	631.42	0.0001
4. *A. canescens* (F.R.1)	0.75	28.43	583.16	0.0003
5. *A. canescens* (F.R.2)	0.85	-31.92	556.12	0.0001
6. *A. canescens* (Santa Rita)	0.86	84.98	557.42	0.0001
7. *A. canescens* (Rincon)	0.93	23.24	328.22	0.0001
8. *A. cinerea*	0.59	239.31	305.74	0.0036
9. *A. glauca*	0.78	244.42	640.17	0.0001
10. *A. halimus*	0.64	289.34	362.59	0.0019
11. *A. lentiformis*	0.83	57.78	414.40	0.0001
12. *A. nummularia*	0.78	280.99	655.94	0.0001
13. *A. paludosa*	0.75	-115.26	1486.19	0.0003
14. *A. rhagadioides*	0.96	-175.48	805.34	0.0001
15. *A. semibaccata*	0.28	721.37	477.86	0.0768
16. *A. undulata*	0.81	-22.49	601.54	0.0022

$Y = a_{1..16} X + b_{1..16}$ [where Y = DM(g/plant) and X = volume(m^3)]

The growth form of these three accessions might not be suitable for the measurement method that was used to determine the dimensional measurements or the number of observations of only 12 for each accession was not enough for this model. The relationship between DM production and plant volume is also illustrated in Figures 2 and 3 for *A. semibaccata* and *A. rhagadioides* respectively. From these figures it is evident that the ranges of both volume (0.4 - 1.95 m^3) and DM production (800 - 2400 $g.plant^{-1}$) of *A. semibaccata* were relatively low compared with the volume of *A. rhagadioides* which ranged between 0.2 - 4.6 m^3 and DM production which ranged between 0 and 3800 grams per plant. These differences in variation possibly contributed to the differences between r^2 values (*A. semibaccata* = 0.28 and *A. rhagadioides* = 0.96) of these two species.

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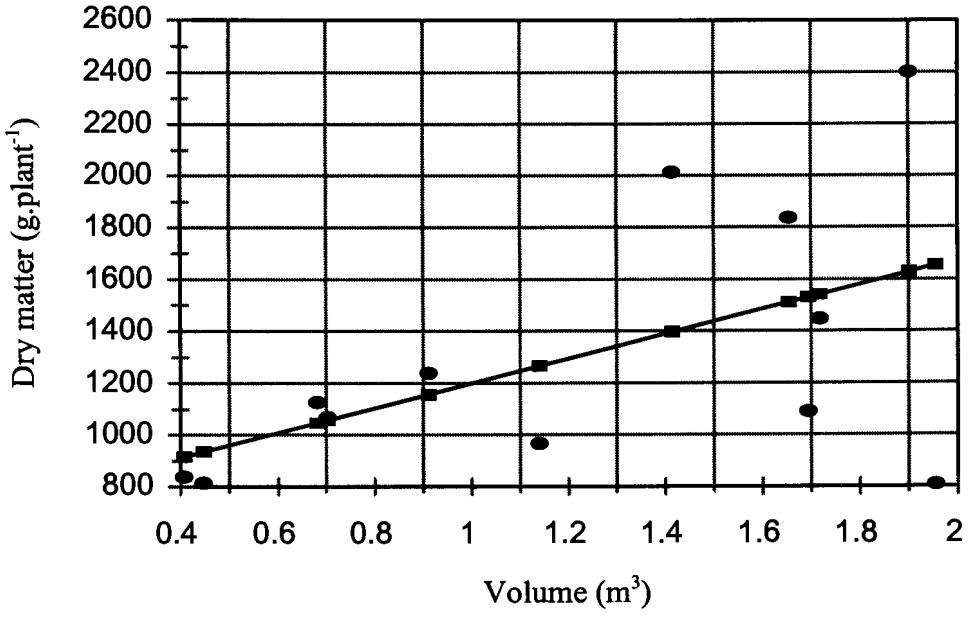

Figure 2 Relationship between total aerial DM production and volume of *A. semibaccata*

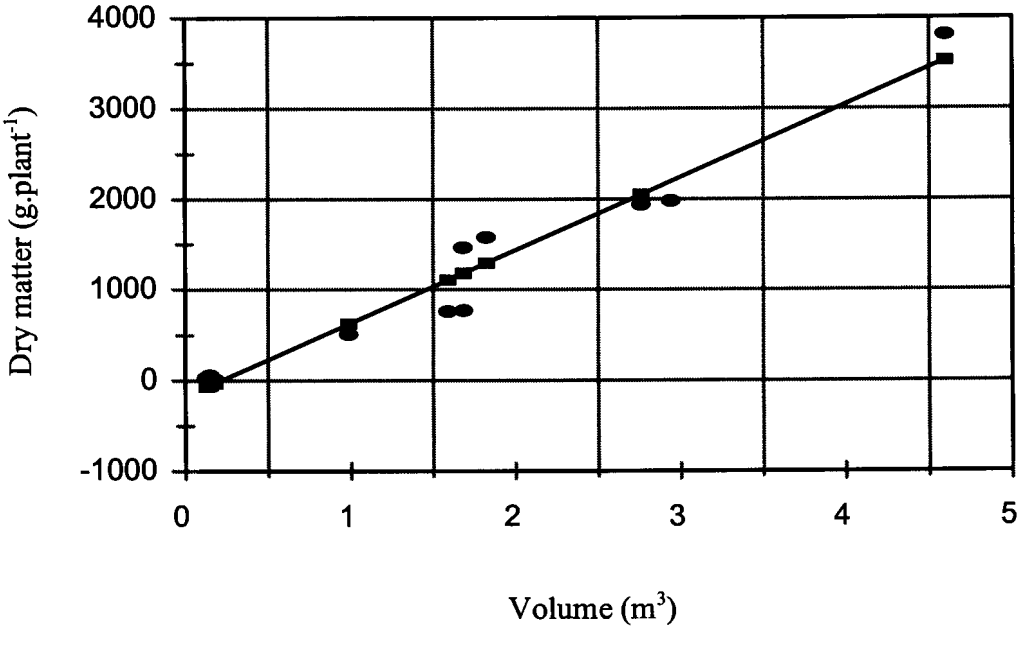

Figure 3 Relationship between aerial DM production and volume of *A. rhagadioides*

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Model 3

For the third model, the shrub height and diameter of each of the 16 accessions (12 observations per accessions, except *A. undulata* with only eight observations) were related to its DM production [$Y = c_{1..16} X + d_{1..16} Z + b_{1..16}$]. The regression parameters of the 16 regression analyses are presented in Table 3. With this model r^2 values of 0.74 to 0.90 were obtained for most accessions, which were more or less the same as for Model 2. For *A. cinerea* ($r^2 = 0.76$)and *A. halimus* ($r^2 = 0.75$) DM production could be estimated more accurately with this model than with Model 2. The r^2 value for *Atriplex semibaccata* was, however, still very low (0.37). For this model the r^2 value for *A. paludosa* was lower (0.66) than that obtained with Model 2 (0.75).

Table 3 Results of the regression between the height and width (diameter) of each accession and its DM production

Accessions	r^2	Intercept (b)	Slopes (c)	Slopes (d)	Significance level
1. *A. amnicola*	0.86	-1763.89	2558.75	720.16	0.0001
2. *A. breweri*	0.85	-1183.63	901.69	1039.91	0.0002
3. *A. canescens* (Austr.)	0.90	-950.21	267.16	1125.68	0.0001
4. *A. canescens* (F.R.1)	0.74	-535.79	-2.23	929.46	0.0023
5. *A. canescens* (F.R.2)	0.87	-1347.49	1821.60	345.82	0.0001
6. *A. canescens* (Santa Rita)	0.89	-1344.66	-122.31	1952.61	0.0001
7. *A. canescens* (Rincon)	0.87	-211.26	-214.24	622.51	0.0001
8. *A. cinerea*	0.76	-923.57	2016.70	168.13	0.0017
9. *A. glauca*	0.90	-1997.60	486.15	1755.30	0.0001
10. *A. halimus*	0.75	-838.81	394.32	988.26	0.0020
11. *A. lentiformis*	0.83	-494.15	335.52	613.92	0.0003
12. *A. nummularia*	0.89	-884.54	-510.44	2251.30	0.0001
13. *A. paludosa*	0.66	-470.55	-483.56	1365.77	0.0081
14. *A. rhagadioides*	0.83	-1370.31	603.60	1381.20	0.0004
15. *A. semibaccata*	0.37	73.87	-630.23	631.15	0.1264
16. *A. undulata*	0.85	1013.26	1365.14	531.17	0.0093

$Y = c_{1..16} X + d_{1..16} Z + b_{1..16}$ [Y = DM production, X = height(m) and Z = width(m)]

Model 4

For the fourth model, all the accessions (188 observations) were used together in one model, where shrub volume was related to DM production, but each accession had its own intercept while the slope was the same for all accessions [$Y = eX + b_{1..16}$]. The results are presented in Table 4. The r^2 value for this model was 0.80 which means that 80% of all variation in DM production was accounted for.

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Table 4 Results of the regression between the volume of each accession and its DM production. The slope and r^2 value for all 16 accessions were chosen to be the same, while the intercepts differed from each other.

Accessions	Intercept (b)	Slope (a)
1. *A. amnicola*	210.09	554.19
2. *A. breweri*	167.99	554.19
3. *A. canescens* (Austr.)	52.05	554.19
4. *A. canescens* (F.R.1)	58.08	554.19
5. *A. canescens* (F.R.2)	-28.99	554.19
6. *A. canescens* (Santa Rita)	92.93	554.19
7. *A. canescens* (Rincon)	-136.82	554.19
8. *A. cinerea*	-164.64	554.19
9. *A. glauca*	378.47	554.19
10. *A. halimus*	-117.97	554.19
11. *A. lentiformis*	-75.60	554.19
12. *A. nummularia*	442.51	554.19
13. *A. paludosa*	276.18	554.19
14. *A. rhagadioides*	215.89	554.19
15. *A. semibaccata*	628.25	554.19
16. *A. undulata*	14.08	554.19

$Y = eX + b_{1..16}$ [Where Y = DM (g/plant), X = volume (m^3) and e = 554.19]; $r^2 = 0.80$; P = 0.0001

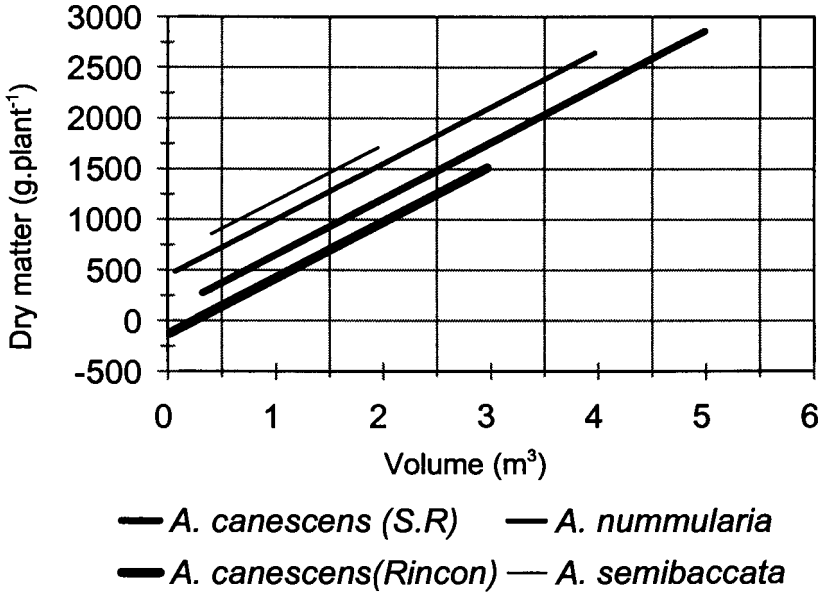

Figure 4 Relationship between total aerial DM production and volume of four *Atriplex* accessions. The slopes are the same but with different intercepts

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In Figure 4 the relationships between DM production and plant volume for four of the accessions (*A. canescens* Santa Rita, *A. canescens* Rincon, *A. nummularia* and *A. semibaccata*) are plotted.

When considering the prediction equation obtained by fitting Models 2 and 3 on the data of individual *Atriplex* accessions, it is evident that the accuracy of the regressions for some accessions was relatively low. For example, the r^2 values for *A. semibaccata* were 0.28 and 0.37 of Models 2 and 3 respectively. This accession had a totally different growth form than any of the other accessions. Its common name of creeping saltbush is a good description of its creeping growth form, with heights seldom higher than 0.3 meters and diameters of up to three meter. *Atriplex semibaccata* also proved to have the highest DM production of all the accessions. There might be some other measurements, such as leaf density, or number of shoots per unit area, that could be incorporated to develop a more accurate model for DM simulation of *A. semibaccata*.

The total harvested DM was also divided into edible components (leafs and edible twigs) and inedible components (woody twigs). Two regression were done, one where edible DM was related to plant volume and the second where inedible DM was related to plant volume. The r^2 values were 0.53 for the edible DM and 0.59 for the inedible DM. These relatively low r^2 values indicates that edible and inedible DM were not highly correlated with plant volume. In Table 5 the edible and inedible DM components are illustrated as percentage of the total DM production for each of the 16 accessions, on each of the three time periods over which they were harvested. It is suggested that this table could be used after prediction of total DM production (using one of the four discussed models) to determine more or less the structural composition of the predicted total dry matter.

The plant age of the 16 accessions varied from eight to 15 months. If any of the models, discussed above, are applied to plants older than 15 months, the results might not be accurate.

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Table 5 Structural composition, as a percentage of total dry matter production, of the 16 *Atriplex* accessions at three different time periods after planting. Edible parts are the leafs and young twigs and inedible parts are the older, woody twigs.

Accessions	8 months		11 months		15 months	
	Inedible	Edible	Inedible	Edible	Inedible	Edible
			%			
A. amnicola	9	91	24	76	26	74
A. breweri	9	91	28	72	40	60
A. canescens (Austr.)	14	86	34	66	51	49
A. canescens (F.R. 1)	3	97	30	70	33	67
A. canescens (F.R. 2)	3	97	35	65	31	69
A. canescens (Rincon)	0	100	36	64	47	53
A. canescens (S. R.)	14	86	42	58	50	50
A. cinerea	4	96	9	91	18	82
A. glauca	10	90	27	73	20	80
A. halimus	18	82	32	68	48	52
A. lentiformis	6	94	37	63	43	57
A. nummularia	13	87	47	53	52	48
A. paludosa	13	87	20	80	22	78
A. rhagadioides	2	98	18	82	13	87
A. semibaccata	0	100	1	99	1	99
A. undulata	*nm	nm	21	79	30	70

*not measured (nm)

CONCLUSION AND RECOMMENDATIONS

All four the models investigated in this study have the potential to be used for the prediction of DM production of the 16 *Atriplex* accessions. Dry matter production of *Atriplex semibaccata*, with its different growth form, could not be estimated accurately and it is suggested that another more reliable model be developed for this plant.

It would be better to develop a model for each of the 16 accessions than one model that will work for all sixteen, as was the case with Model 1 where only 72% of the variation could have been account for by the model. To do this more than 12 plants of each accessions should be harvested to obtain more reliable prediction equations. It would be more acceptable if the r^2 values of all the accessions were above 0.90, as was reported by Wilke (1980), Murray & Jacobson (1982) and Thomson *et al* (1998). Abdul-Halim, Al-Badri, Yasin & Ali (1990) studied eight of the 16

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Atriplex accessions studied in this trial and found that DM production was highly correlated to plant height and diameter with a r^2 value of 0.94. This correlation was for one model which included all eight *Atriplex* species with up to 33 plants per species.

In this trial the volumes of all 16 accessions were determined as the volume of a cylinder as was also done by Wilke (1980), while Murray & Jacobson (1982) used different mathematical formulas to determine the volumes for shrubs with different shapes, like: right cylinder, elliptical cylinder, sphere and spheroid. Thomson *et al* (1998) suggested that for younger and smaller shrubs a cylinder would be a more appropriate model to predict aerial biomass, whereas in older shrubs an ellipsoid is more appropriate. This was also visible in this trial in Figure 1 where most of the dots on the graph, representing the younger (lower DM production and smaller volume), were close to the regression line, while the older plants were positioned further away from the regression line. More accurate models might be developed if other shapes than a cylinder are tested for each of the 16 accessions.

The different aerial dimensions of the different accessions together with older plants needs further study. Another factor that also requires further study is to develop a model to simulate the regrowth or DM production of previously browsed shrubs.

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CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

With respect to the dry matter production, structure and nutritive value of the 16 *Atriplex* accessions evaluated, it can be concluded that major differences were evident. Accessions varied in growth form, from a creeping growth form (*A. semibaccata*) to a prostrate growth form (*A. amnicola*) to an upright growth form (*A. nummularia*). The relative size of the accessions indicate the need for espacement trials to determine optimal espacement for the different species under different ecological conditions. With the exception of *A. breweri* and *A. lentiformis*, all 16 accessions were relatively tolerant of a harvest height of 10 cm. In all 16 accessions the percentage leaves decreased over time while the percentage twigs increased. The percentage edible twigs also decreased while the inedible twigs increased over time. The total edible material, 15 months after planting, ranged from 0.71 $t.ha^{-1}$ (*A. canescens* Rincon) to 5.78 $t.ha^{-1}$ (*A. rhagadioides*). The accessions producing the highest yiels of CP per hectare, were *A. amnicola*, *A. canescens* (S.R.), *A nummularia* and *A. rhagadioides*, which all produced 550 kg. $CP.ha^{-1}$ or more.

In vitro DM digestibility was much higher for leaves than for edible twigs, ranging from 69% (*A. nummularia* and *A. semibaccata*) to 81% and 83% (*A. amnicola* and *A. rhagadioides* respectively) in the leaves and from 17% and 19% (*A. canescens* S.R. and *A. canescens* Austr. respectively) to 52% (*A. nummularia*) in the edible twigs. The mean, for edible twigs and leaves, ranged from 42% (*A. glauca*) to 60% (*A. nummularia*). Edible twigs had higher NDF values than leaves. The accessions with the highest NDF content in the edible component were *A. canescens* (F.R.1) at 62% and *A. glauca* and *A. semibaccata*, at 61%. The accessions with the lowest NDF content in edible material, were *A. paludosa* (46%), *A. nummularia* (48%) and *A. canescens* (S.R.) (49%). Crude protein was much higher in leaves than in edible twigs. *Atriplex glauca* and *A. semibaccata* had the lowest CP content, at 8% and 9% respectively, while *A. nummularia* on 15% and *A. canescens* (S.R.), *A. halimus* and *A. rhagadioides*, all on 13%, had the highest CP content. *Atriplex canescens* (Rincon and Austr.) and *A. undulata* had the lowest ash content, at 15%, while *A. paludosa* (25%) had the highest ash content. *Atriplex canescens*

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(Santa Rita) had the lowest sodium content at 0.05%, while *A. nummularia* (2.72%) had the highest sodium content. *Atriplex paludosa* had the lowest OM value in edible material, at 75%, while the other accession were grouped around 83%.

With respect to DM production and nutritive value *A. nummularia*, *A. canescens* (S.R.), *A. rhagadioides* and *A. amnicola* were the best accessions, while *A. semibaccata*, *A. glauca* and *A. canescens* (Rincon) were the weakest. It is, therefore, recommended that when shortlisting such species for use as drought fodder, priority be given to *A. nummularia*, *A. canescens* (S.R.), *A. rhagadioides* and *A. amnicola*. These accessions should be further tested and compared as fodder crops in larger trials. In contrast it is suggested that *Atriplex glauca* (prostrate growth form) and *A. semibaccata* (creeping growth form) should be incorporated in rehabilitation trials on degraded land and for soil erosion control. From this study *A. canescens* (Rincon), *A. breweri* and *A. undulata* is of little to no value. The IVDMD and CP contents of *Atriplex* accessions should also by studied more intensively in future. Some researchers reported that IVDMD values could be misleading because of high contents of soluble salts in the ash of *Atriplex* species. The occurrence of non protein nitrogen may also result in a misleading CP content for *Atriplex* species.

Rather high standard deviation values indicated that number of replicates were too low. This was also enhanced by the clear genetic variation between individual plants of the same species. It is suggested that in future more plants per species should be harvested for evaluation, to minimize the influence of genetic variation.

With respect to adaptation to different ecological areas and in particular to arid and semi-arid conditions, the performance of accessions under stress conditions is of critical importance. The survival rate at Bloemfontein averaged only 41%, while it ranged from 69% to 87% at the other locations. Survival rates were not only influenced by ecological and climatological conditions but also by the management practices at the different sites. The accessions with average survival rates lower than 69% were *A. amnicola*, *A. cinerea*, *A. paludosa*. *A. rhagadioides* and *A. undulata*. With the exception of *A. undulata* these were the more herbaceous accessions. Relative plant volume of all accessions were larger at Pretoria (higher average rainfall) and smaller at Pofadder and Kathu (lower average rainfall). Plants at Mier were also larger than at the latter two location as they were irrigated regularly by the farmer. Accessions at Kathu and

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Mier had a better nutritive value than Pretoria and Pofadder. This can, however, be explained as the samples contained mainly young, herbaceous plant material, which would result in lower NDF values and higher CP and IVDMD values. The material from Mier and Kathu, therefore, had higher sodium and ash values and lower OM values than that from Pretoria and Pofadder. The four accessions that were best adapted at each location, according to survival and nutritive value, were: *A. amnicola*, *A. canescens* (S.R.), *A. halimus* and *A. nummularia* at Pretoria; *A. amnicola*, *A. canescens* (S.R.), *A. lentiformis* and *A. nummularia* at Kathu and Mier; *A. canescens* (S.R.), *A. glauca*, *A. lentiformis* and *A. nummularia* at Pofadder; and *A. canescens* (S.R.), *A. glauca*, *A. halimus* and *A. nummularia* at Bloemfontein. These findings were, however, based on results after only one year. Longevity would also be a strong consideration and follow up observations should be made. At this stage it would appear that over the short term *A. canescens* (S.R.), *A. lentiformis* and *A. nummularia* would do the best in the very dry areas, while all five *A. canescens* accessions, *A. glauca*, *A. halimus* and *A. nummularia* had the widest adaptation. The latter should therefore enjoy the largest inputs in future programmes. It is suggested that fewer accessions be planted in larger plots for further adaptation studies. It is also recommended that planting date and season should be synchronised and that sample taking and sample composition should be the same for all locations.

With respect to palatability of different accessions, as indicated by the selection of *Atriplex* species by grazing sheep it was found that *A. amnicola*, *A. canescens* (S.R.), *A. cinerea*, *A. rhagadioides* and *A. semibaccata* had the highest palatability, while *A. canescens* (Austr.), *A. canescens* (Rincon), *A. paludosa* and *A. undulata* were the least palatable. *Atriplex halimus* and *A. nummularia* had a low reduction in volume but were ranked as accessions with high palatability according to direct observation. This ranking was confirmed by the photographic evaluation which indicated that most of the leaves were stripped from the twigs. When the plants were measured for height and diameter (to determine volume), measurements of the amount of leafy material, which formed part of the plants total volume, were not incorporated. The more palatable accessions should be planted in larger plots with more plants of each species. Such plots should allow the trial to proceed for a longer period, which will give the animals more time for selection and it should be grazed in each of the four seasons to evaluate the effect of season on palatability. Different sheep and goat breeds should also be used during further palatability

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studies as their preferences might vary.

The oesophageally fistulated sheep selected diets ranging in CP content from 11% to 18%, NDF values from 32% to 44%, and IVDMD values from 60% to 74%. The nutrient content, of selected diets, had been expected to decline over time, but did not. There were, however, variations in the quality of selected diets between the four paddocks. These diets were more nutritious than that harvested as edible material by hand, indicating that too thick twigs (up to four millimetres) were allowed as part of the edible component, during hand harvesting. In future the twig diameter should be measured after grazing to determine the diameter acceptable for different animals. In future this study should be repeated on the four most palatable plants to determine the quality of selected diets from only four accessions instead of 16 accessions.

In future more time should be spent on selecting suitable ecotypes within *Atriplex* species. Larger plots, containing single species should be planted, and screened for individuals with a specific set of criteria. Once suitable individuals have been selected, for example as palatable plants, they should be multiplied vegetatively. These multiplied plants should then be planted far away from other plants of the same species to allow them to pollinate each other. The seed should be harvested, planted in new plots, and selection of palatable plants should be done again. The selected material should be propagated vegetatively and the same procedure should be followed. This should continue until the seed is known to yield plants that are relatively homogenous, concerning palatability

Concerning *in vitro* propagation of elite *Atriplex* shrubs, the best disinfestation procedure gave survival rates ranging from 71% to 100% for *A. nummularia*. It is recommended to first produce greenhouse grown mother plants of selected material from stem cuttings, before using it for micropropagation. This will limit contamination during the disinfestation stage. The best medium for multiplication of *A. nummularia* was the MS basal medium with 0.20 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA, while the MS basal medium, without any plant growth regulators, was the best for elongation and rooting. The best medium for shoot multiplication and elongation for *A. canescens* was the MS basal medium with addition of 0.20 $mg.l^{-1}$ BA and 1.0 $mg.l^{-1}$ NAA, while a medium with 0.05 $mg.l^{-1}$ BA and 0.05 $mg.l^{-1}$ NAA was good for elongation and rooting. Average survival rate after acclimatization of *A. nummularia* was 89%. Micropropagation of *A. canescens* was not satisfactory. It is suggested that other media, like ½-strength MS basal

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medium, should be tested with other plant growth regulators, like gibberellic acid. A suitable rooting medium for *A. canescens* should also be tested for. The micropropagation procedure for *A. nummularia* also needs some refinements to enhance the multiplication rate.

For the vegetative propagation of *Atriplex nummularia* by stem cuttings, the average percentage rooting was higher in spring (47%) than in the autumn (42%) or summer (37%). The new growth plant (50%) had a higher average rooting percentage than the old growth (35%) plant. Average percentage rooting was higher for terminal (63%) than for subterminal (21%) cuttings. The best hormonal treatment was that of 3 $g.kg^{-1}$ IBA. The best results could be expected by using terminal cuttings of the new growth in the late autumn or spring, treated with 3 $g.kg^{-1}$ IBA.

It is recommended that in future different plants of the same species should be used for propagation by stem cuttings, as genetic variation should be taken into account. Different plants of the same species might posses different rooting abilities and if a method is recommended for only one plant it might not necessarily work for all different plants of the same species. Species with a prostrate to creeping growth form, like *A. semibaccata*, *A. amnicola*, *A. undulata*, *A. glauca*, *A. cinerea* and *A. rhagadioides*, might also propagate easily by stem cuttings, as their branches tended to form roots where it touched the soil. It is suggested that vegetative propagation of other promising species also be tested.

For both methods of vegetative propagation several plants of the same species should be multiplied to maintain genetic variability, to reduce the vulnerability of the multiplied plants to pests and diseases. In future, these two methods should be compared to determine which is the more economical. The advantages of *in vitro* propagation is however, that many homogenous plants can be produced in a short time and out of season. Such cultures can be preserved for further studies and can easily be transferred from one country to another, as sterile cultures. A practical proposition would be to increase the numbers of selected material quickly through *in vitro* propagation and continue propagating it by stem cuttings. With further refinements, large scale vegetative propagation of elite *Atriplex* accessions, should be feasible.

All four tested models were acceptable to simulate the DM yield of the 16 *Atriplex* accessions. For Model 1, where all 16 accessions's shrub volumes were related to DM yield in one model, the r^2 value of 0.72 was low but acceptable. For Model 2, where the volume of each accession were related to its DM yield, almost all the r^2 values were above 0.74. *Atriplex semibaccata*

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(0.28), *A. cinerea* (0.59) and *A. halimus* (0.64), were the only accessions with r^2 values lower than 0.74. For Model 3, where shrub height and diameter of each accession were related to its DM yield, with the exception of *A. semibaccata* (0.37) and *A. paludosa* (0.66), all the r^2 values were above 0.74. For Model 4, where all accessions were used in one model, shrub volume was related to DM yield, while each accession had its own intercept but the slope were the same for all, the r^2 value was 0.80. It is, therefore, possible to simulate DM yield of any of the 16 accession by using any of the tested models. Simulating DM yield of *A. semibaccata* would, however, not be possible with these models. The low r^2 values and significance levels, might be caused by the creeping growth form of *A. semibaccata*, which was different from the prostrate to upright growth form of the other accessions. The relatively high leaf percentage might also be a reason as *A. semibaccata* will give a higher DM yield than another accession with the same volume but a lower leaf percentage.

It would be better to develop a model for each of the 16 *Atriplex* accessions than one model for all the accessions. More reliable models could be obtained if more plants per accession are harvested and measured. Only 12 plants were harvested for this study, while other researchers have used 30 plants and more. The different aerial dimensions of the different species together with plants of different ages needs further investigation. Prediction of DM yield from plant height and diameter would be an appropriate non destructive method, to use before a grazing trial, to monitor the amount of DM available. Such models could also be applied to monitor DM yield of plants growing on farms, without damaging the plants.

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