Rooting of buchu cuttings (Genus: *Agathosma*)

By

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature **Date**

Summary

Buchu (*Agathosma betulina* and *A. crenulata*) are grown commercially as an aromatic crop and are endemic to the Western Cape of South Africa. Poor rooting of cuttings have limited the development of superior clones. Under standard mist bed conditions terminal, sub-terminal or basal stem cuttings were taken from March to August. When not treated with an auxin, rooting percentages of between 20 and 25 were obtained. Rooting percentages increased to between 40 and 45 after treatment with 500-1000 ppm indolebutyric acid (IBA). Substituting IBA with naphthaleneacetic acid (NAA) did not improve rooting. There was a tendency for cuttings with fewer than four leaf pairs to give lower rooting percentages.

Plants of *Agathosma betulina* x *A. crenulata,* grown in Paarl, and *A. betulina,* grown in Piketberg, were used as source plants for making cuttings. Paarl plants were shaded with 80 percent shade and Piketberg plants with 60 or 80 percent shade respectively from February to October 2002. Plants in full sun served as a control. Plants were pruned back initially in February and then two months before samples were taken in March, June, August and October at both locations. New shoots were used as cuttings. Terminal cuttings for rooting and for carbohydrate analyses were collected on four different dates (March, June, August and October). Cuttings were treated with 500 ppm indolebuteric acid (IBA) and placed in misting beds with bottom heating (18-25°C) for a period of three months.

Shading reduced rooting of cuttings from the Paarl plants. However, it did not significantly increase rooting of cuttings taken from Piketberg plants. Rooting percentage was the highest in August (43%) for cuttings from sun grown plants in Paarl. No consistent relationship between, respectively, dry mass or carbohydrate content of cuttings and rooting could be established.

Terminal current years' growth, taken from *Agathosma crenulata* x *A. betulina* (hybrid) softwood cuttings, collected in January 2002, were extracted with methanol and fractioned by thin layer chromatography (Silica gel) in isopropanol: acetic acid: water (4:1:1 v/v). The chromatographs were divided in ten fractions and were bio-assayed for a rooting co-factor

with the mung bean rooting test. Extracts from buchu cuttings showed significant activity at the Rf values of co-factor 3. Co-factors 1,2 and 4 do not seem to be present in significant quantities.However, co-factors with Rf values different from previous reported values were present in significant quantities. No inhibition was found in buchu. In fact, all Rf values stimulated rooting.

Opsomming

Boegoe (*Agathosma betulina* x *A. crenulata*) word kommersieël verbon as 'n aromatiese gewas en is endemies tot die Wes-Kaap. Die ontwikelling van superieure klonale materiaal word beperk deur swakbeworteling. Terminale, sub-terminale en basale steggies is gesny onder standaard misbed toestande van Maart tot Augustus. Beworteling was tussen 20 en 25 persent as geen ouksien gebruik word nie. As indolebottersuur (IBS) gebruik word tussen 500-1000 dpm, verhoog die bewortelingspersentasie tot tussen 40 en 45 persent. Die gebruik van naftaleen asynsuur (NAS) in plaas van IBS het nie beworteling verbeter nie. Daar was a tendens dat steggies wat minder as vier blaarpare gehad het 'n verlaging in bewortelingspersentasies gehad het.

Plante van Paarl, *A. betulina* x *A. crenulata,* en Piketberg, *A. betulina,* is gebruik as plantmateriaal vir steggies. Plante in die Paarl was onder 80 persent skadu geplaas en plante in Piketberg onder 60 en 80 persent skadu van Februarie tot Oktober 2002. Plante in volson was as 'n kontrole gebruik. Plante was eers in Februarie teruggesny en dan weer twee mande voor monsters geneem is. Die monsters is in Maart, Junie, Augustus en Oktober geneem in beide liggings. Terminale steggies is vier keer ingesamel (Maart, Junie, Augustus en Oktober) vir beworteling en koolhidraat analises. Die steggies is met 500 dpm IBS behandel. Daarna is die steggies vir drie maande in die misbed geplaas met bodem-verhitting (18-25°C).

Dit is gevind dat die gebruik van skadu die beworteling in Paarl verminder het alhoewel die beworteling in Piketberg nie beduidend beinvloed is nie. Die hoogste bewortelingspresentasies is waargeneem in Augustus (43%) in Paarl van plante wat in vol son was. Geen verband tussen onderskeidelik die droe massa of koolhidraat inhoud en beworteling kon gevind word nie.

Terminale steggies van dieselfte jaar se groei van *Agathosma betulina* x *A. crenulata* (hibried) is in Januarie 2002 ingesamel. Die materiaal is geëkstraheer en gefraksioneer deur dunlaag kromatografie in isopropanol: asynsuur: water (4:1:1 v/v). Die kromograaf is in 10

fraksies verdeel. Die fraksies was bioassaieer vir beworteling ko-faktore met die mungboontjie bewortelingstoets. Die ekstrakte van boegoe het beduidende aktiwiteit by die Rf waardes van ko-faktor 3 getoon. Ko-faktore 1, 2 en 4 is nie in groot genoeg hoeveelhede waargeneem nie. Ko-faktore, wat nie voorheen gevind is nie, is waargeneem in beduidende hoeveelhede. Geen inhibitors is in boegoe gevind nie en al die getoetste ko-faktore het beworteling gestimuleer.

Nihil nimis difficile

To Dad, Mom, Gran:

Your tide of overwhelming love and support has brought me here.

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Contents

1. Introduction

Buchu is one of South Africa's most well known medicinal plants, as it has been tried as a cure for many diseases that afflict mankind (Simpson, 1998). Dried buchu leaves are used as a brandy and vinegar tincture, known as buchu brandy and buchu vinegar. Both products are used as household medicines in South Africa, to remedy infections of the kidney and urinary tract. They are also commonly used as local applications to bruises and for the relief of rheumatic pains (Van Wyk *et al.*, 1997). According to Roberts (1997), some species have an agent that blocks ultra violet light, hence showing a potential use in sunscreen creams.

Besides the favourable medicinal properties of buchu, a great economical importance lies in the extraction of its foliar oils for export. buchu oil is used commercially for its black current flavour and smell, hence as a suitable natural flavourant (Posthumus *et al.*, 1996; Roberts, 1997; Simpson, 1998). Herein probably lies the greatest economic future of buchu, following the global trend towards the use of natural products in consumable commodities. At a low concentration of 5 ppm, buchu oil is used to enhance and fix the inherent flavour of mango, tropical and berry flavourants (Masciano *et al.*, 1994). Buchu oil is also used in perfumes, cosmetics and soaps.

Poor rooting of buchu cuttings has limited the progress in developing superior clones. This thesis report on some of the factors that affect rooting of buchu cuttings as well as the relationship with carbohydrate reserves and rooting co-factors in the cuttings. This review focuses on some of the botanical aspects of buchu, as well as on the current propagation and cultivation methods employed today. Furthermore, the current knowledge on oil composition will be discussed.

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2. Buchu (*Agathosma)*: A Literature Review

2.1 Taxonomy and geographical distribution

Buchu belongs to the family Rutaceae and the genus *Agathosma*. The family Rutaceae is the ninth largest in the Cape Floral Kingdom, consisting of 150 genera of which 15 are found in the Cape region. However, only six of the 15 genera are endemic to this area including some of the economic important buchu species.

The name *Agathosma* stems from two Greek words "agathos" and "osme" meaning "good" and "smell", respectively (Spreeth, 1974). Pillans (1950) identified two buchu species based on differences in the fruiting body and leaf shape. The leaf of *A. betulina* was said to be broader at the tip of the leaf than the bottom half, whereas *A. crenulata* is just the opposite, being broader in the bottom half of the leaf. Spreeth (1974), rather describes the *A. betulina* leaf as rounded at the apex and bent back at the tip with a length: width ratio of 2:1. *A. crenulata* is also rounded at the apex but the tip does not bend back and has a length: width ratio of 2:3 (Spreeth, 1976).

Agathosma betulina (Berg.) Pillans (round leaf) and *A. crenulata* (L.) Hook (oval leaf) were formerly known as *Barosma betulina* and *B. crenulata* until they were reclassified by Pillans (1950). Pillans (1950) recognised two commercially important buchu species, namely *A. betulina* and *A. crenulata*. Spreeth (1974) recognises a third economically important species, *A. serratifolia* (long leaf), which was thought to be a hybrid of *A. crenulata* (Van Wyk *et al.*, 1997). Goldblatt and Maning (2000), however, recognises *A. crenulata* and *A. serratifolia* as two different species

The occurrence of the three species differs geographically. *A. betulina* is found on the rocky sandstone mountain slopes from Cedarberg to Tulbagh. *A. crenulata* is found from Ceres to Swellendam on the middle slopes of mountains and valleys. The long leaf (*A. serratifolia*) occurs on the south facing mountain slopes from Caledon to Riversdale. . However, today a new hybrid is seen in the field. The planting of the two species out of

there geographical regions has result in the cross pollentation *of A. Betulina* and *A. crenulata.*

2.2 Morphology

Buchu is a perennial shrub, reaching a height of 2 to 2.5 m. The leaves are about 20 mm long. They are opposite and almost sessile. The leaf margins are bordered with sharp serrations, with oil glands situated at the base of each serration. The underside of the leaf also has numerous oil glands.

Agathosma betulina flowers are white or purplish pink whilst *A. crenulata* has white or mauve flowers (Goldblatt and Maning, 2000). The flowers are born single in the axis of the distal leaves of the stem. The flowers consist of 5 petals and 5 sepals with a hypogenous ovary (Van Wyk and Gericke, 2000). The fruit consists of five chambers (carpels) and when mature, each contain a single oblong, glossy black seed of 2 - 4 mm in size.

2.3 Propagation

In the past buchu was only harvested from the wild and to a certain extent this practice continues. However, in recent years there has been a strong tendency towards commercial buchu plantations, which requires large quantities of suitable plant material.

According to Von Wielligh (1913), buchu can be propagated either by seeds or cuttings. Propagation by seed is still the main method employed by the industry, as shoot cuttings have a fairly low rooting success. However, it is difficult to collect large enough quantities of viable seeds effectively. Furthermore, seed propagation results variability in plant material. There is thus a need for improving the rooting potential of buchu cuttings, which would allow us to take advantage to be taken of superior clonal material, especially in terms of high oil content and quality.

2.3.1 Seed propagation

2.3.1.1 Seed collection

Flowering occurs from June to November (Goldblatt and Maning, 2000) and the seed-ripens from January to February. Due to the long flowering period, the seeds ripen fairly irregularly, causing difficulty in of determining the optimal time for seed collection.

Temperature extremes and general condition of the plants at seed set have a major influence on the amount of seeds produced. Plants under minimal stress tend to produce more seeds. Very cold or extremely hot periods may significantly lower the percentage seed set. There appears to be genetic variability, as plants grown under the same conditions produce different amounts of seeds. Older plants also show a higher seed set than young ones (unpublished data, Louisa Blomerus, 2002).

Once the seeds ripen naturally, the fruit chambers dehisce and the seeds are scattered (Simpson, 1998). According to Blomerus (2003), there are three methods of seed collection currently employed in the industry.

1. Seeds can be hand picked before the chambers dehisce naturally. However, using this method only a maximum germination percentage of around 19% has been reported. The low germination percentage can probably be ascribed to the difficulty in determining the physiological maturity of the seeds at harvest. Thus, the picking of viable seeds largely depends on the skill of the picker.
2. Alternatively, the whole branch with capsules can be removed and allowed to dry slowly till the capsules dehisce on their own. The shoots should be placed in a sun-protected area, as seeds should not dry too fast.
3. As a third method, the shoots can be enclosed in cheesecloth or wire gauze bags to catch the seeds when the fruit chambers dehisce naturally. This method results in the collection of seeds with a germination percentage as high as 90%. Viable seeds can be identified as those seeds that sink in distilled water (Blommaert, 1972; Simpson, 1998).

2.3.1.2 Seed storage

Seeds should not be stored for long periods, as the germination success is said to decline. The seeds must be kept dry during storage and can be stored in airtight containers, in a cool and dry place for short periods of time. If slightly longer storage is required, the seeds should be placed into a refrigerator at 4° C. However, the percentage germination of seeds with high oil content is preserved better if stored at 0° C [1](Pers. communication Louisa Blomerus).

2.3.1.3 Seed germination

The optimum time to plant sow is in March (autumn), before the soil gets too cold (Phillips, 1917; Werner, 1949; Roberts, 1997). Compton and Mathews (1921) planted seeds in a deeply ripped or trenched soil in March to April after good rains. The seeds were planted into the soil about 10 cm apart and at a depth of 2.5 cm, with three seeds per planting hole. During the following year, the empty spaces, due to unsuccessful germination were filled. Some make use of seedbeds for germination and the young seedlings will remain there until ready for transplantation into the plantation.

Matthews (1919) found that seeds have a variable germination success of 20 percent in one year and up to 80 percent in another. Compton and Mathews (1921) stated the average as being between 40 to 50 percent. In general, the success of germination can be described as poor, and pre-treatment of buchu seeds to improve germination, is required. Furthermore, with a pre-treatment, germination can be done all year round, instead of being restricted to the autumn period.

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[1] Blomerus., L., 2003. Agricultural Research Council. Fynbos Unit. Private Bag X1, Elsenburg, 7607. South Africa.
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Due to the hard outer seed coat, seeds require scarification to improve germination (Brown, 1999). Blomaert (1972) used 20 minutes of dry heat at 80°C to break the seed shell, which ultimately improved germination. An alternative method is to soak the seeds in sulphuric acid for 45-60 minutes after which the seeds are thoroughly washed. The scarification process is followed by a 24 hour pre-soak with Kirstenbosch Smoke Primer solution (Brown, 1999).

2.3.2 Propagation by cuttings

Von Wielligh (1913) found that cuttings rooted when taken in autumn (when plants are dormant) from wood of the previous year's growth. The cuttings were rooted in river sand without nutrients added. They were covered with a bell glass or tumblers, to retain the moisture content. The tumblers were removed every morning and dried and then replaced. [2] (Pers. communication Louisa Blomerus), reported a rooting percentage of 67% for cuttings taken in August. Two parts Canadian peat:1 part sand were used as a rooting medium and cuttings were treated with 2000 ppm IBA. Cuttings were rooted under standard mist-bed conditions. The mist-irrigation was set to 3 minutes once an hour.

2.4 Cultivation

Little has been reported on the cultivation of buchu. There are still many questions that need to be answered regarding irrigation scheduling, fertilisation, pruning methods and harvesting of the crop.

Seedlings should be planted out when they are about 5 cm tall. Transplanting should be done before Spring (September). The survival rate of transplants range from 10% to

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100% depending on the after-care of the seedlings and also the quality and vigour of the seedlings [3](Pers. communication Louisa Blomerus).

Seedlings can also be planted out in Autumn after it has rained. Plants are cultivated under dry land or drip irrigation (personal observation). At a year old the plants are pruned back to 8 cm from the ground to promote branching and there by increase the complexity of the bush. The following year, the bush is cut back again to just above the previous years' cut. The bush will slowly increase in complexity over the years (Compton and Mathews, 1921).

The first crop can be harvested in the third year of cultivation. Harvesting of buchu is done in one of two different ways. The first method is that of harvesting the shoots a few centimetres above the previous years cut, again resulting in increasing the complexity of the bush. Alternatively, the shoots can be harvested every second year, after which the plant is cut down to ground level. This method allows the farmer to harvest seeds in the alternate year.

2.5 Oil composition

The oils produced by buchu plants consist of volatile oils that have been extracted from buchu (Lamparsky, and Schudel 1971; Endenburg, 1972; Kaiser, *et al.*, 1975; Blommaert, and Bertel, 1976; Lawrence, 1976; Posthumus, *et al.*; 1996 and Collins, *et al.*, 1996). The six major components of buchu oil are d-limonene, d-menthone, l-isomenthone, l-pulegone, Ψ-diosphenol and diosphenol (the so-called buchu camphor) (Endenburg, 1972; Kaiser, *et al.*, 1975; Blommaert and Bertel, 1976; Posthumus, *et al.*, 1996 and Collins, *et al.*, 1996) (Figure 1).

These form part of the diverse group of compounds known as terpenes, include primary as well as secondary metabolites. The primary terpenoids include sterols,

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carotenoids, growth regulators, and the dolichols, quinones, and protiens. These compounds are essential for the growth and development of the plant. The secondary metabolites include monoterpenes, sesquiterpenes, and diterpenes. These metabolites are not directly involved in growth and development but play a role in the interaction of the plant and its environment i.e. plant-plant, plant-insect or plant-pathogen interactions (Jones, *et al.*, 1995). The specific reason for the oil production in the buchu plant is unknown.

Terpenes are mainly used for culinary purposes, as pharmaceuticals and in perfumes. Mono- and sesquiterpenes are major constituents of essential oils and are used mainly in perfumes, flavourants, pharmaceuticals or solvents (Runeckles and Marry, 1973).

Lamparsky and Shundel (1971), discovered the cis-trans-8-mercapto-p-menthan-3-one which was said to be responsible for the characteristic odour of buchu. The component 8-mercapto-p-menthan-3-one is found in low concentrations (< 3%) in buchu and is responsible for the blackcurrant flavour and smell (Posthumus *et al.*, 1996, Collins *et al.*, 1996).

The two species' and their hybrids differ in oil content and quality. The oil content depends on the species (genetics), environmental conditions, seasonal variation, physical factors, cultivation practices, extraction method and the method used in oil analysis.

A number of the oils are commonly found in other oil-producing plants. Limonene is found in high concentrations in the peel of a number of lemon cultivar (70-76.5%) (Usai, *et al.*, 1996). Menthone, iso-menthone and pulegone are found in the *Mentha* genus (Stengle and Stahl-Biskop, 1966). There are also a few rare bi- and trifunctionalised monoterpenes that are characteristic to buchu. These include the diosphenols, hydroxylated diosphenol and several hydroxy-menthones.

The quantity and type of oils present in buchu can be used for the identification of *A betulina* and *A. crenulata* and their hybrid. *A betulina* has a pulegone content of <4.5% and *A. crenulata* with >31% (Collins, *et al.*, 1996). Diosphenol was used in the identification of buchu by Blommaert *et al.* (1976) as it was found that (Ψ) diosphenol and diosphenol is

only found in *A betulina* buchu (Posthumus, *et al.*, 1996). Diosphenol has been found in trace amounts in *A. crenulata* by Collins *et al.* (1996), and in low concentrations (1%) by Kaiser, *et al.* (1975). The hybrid has an intermediate amount of pulegone and diosphenol. *A betulina* has a slightly higher 8-mercapto-p-menthan-3-one and a lower 8-acetyithio-p-menthan-3-one content than *A. crenulata.* The opposite is true of *A. crenulata* (Kaiser, *et al.*, 1975).

The oil is extracted by hydro-distillation using the Clevenger like apparatus and analysed with gas chromatograph (Endenburg, 1972; Collins, *et al.*, 1996 and Posthumus, *et al.*, 1996).

The main essential oil components of *A. betulina* are diosphenol (22.3%), isomenthone (19.91%), Ψ-diosphenol [Diosphenol] (18.58%), limonene (11.64%) and menthone (9.82%). Two chemotypes have been recognised in *A. betulina.* The diosphenol chemotype has a high diosphenol (<12%) and low isomenthone (<29%) content. The isomenthone chemotype has a high isomenthone (>31%) and low diosphenol (<0.14%) content (Collins, *et al.*, 1996). *A. crenulata* contains pulegone (53.75%), limonene (11.7%) and trans-8-acelylthio-p-menthan-3-one (6.83%). The main components of the hybrid are isomenthone (33.42%), limonene (21.96%) and pulegone (15.75%) (Posthumus, *et al.*, 1996).

Seasonal variations occur in composition and yield of the oils of both species. Endenburg (1972), found that *A. crenulata* reached its highest oil yield of 3.87% in October with two smaller peaks in April (3.74%) and July (3.73%). *A. betulina's* highest oil content occurred in February (3.87%) and in June (3.63%).

In *A. betulina* diosphenol peaked in March (31.9% of the total fraction measured) followed by Ψ-diosphenol (25.9% in March), limonene (25.1% in June), pulegone (11.5% in October) and menthone (6.2% in September). The highest oil yield in *A. crenulata* is pulegone followed by limonene and isomethone. The pulegone content in the leaves peak in January (69.3%), limonene 18.6% in May and isomethone 12.8% in October.

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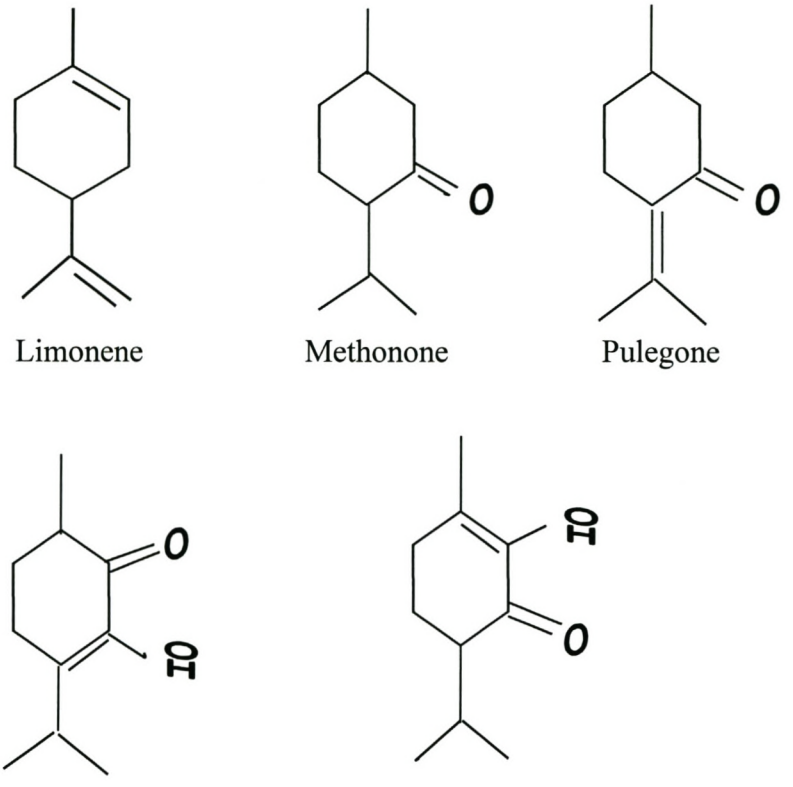

Limonene Methonone Pulegone

psi-Diosphenol (Buchu camphor) Diosphenol

Figure 1: Structures of the major oil compounds found in *A. crenulata* and *A. betulina* (Stengle and Stahl-Biskop, 1966, Endenburg, 1972, van Wyk *et al.*, 1997, Fuchs *et al.*, 2001)

Table 1:The difference in leaf morphology of three Buchu species.

	A. betulina	*A. crenulata*	*A. serratifolia*
Length of petiole in mm	1.0	2.0	1.5
Average length of lamina	20.4	22.9	34.9
Average width of lamina	12.9	10.9	6.5
Point of lamina	rounded bent back	rounded	pointed

Source: Spreth 1974..

Table 2: Average monthly yield from Buchu leaves (in mm/100g dry matter).

Month	*A. betulina*	*A. crenulata*
Jan	3.20	3.52
Feb	3.66	3.83
Mar	3.33	3.33
Apr	3.74	3.53
May	3.65	3.00
Jun	3.44	3.63
Jul	3.73	3.28
Aug	3.40	3.38
Sep	3.51	2.48
Oct	3.87	3.30
Nov	3.26	2.87
Dec	3.14	3.10
Ave. % oil	3.49	3.86

Modified from Endenburg (1972).

3. Paper I-

Rooting of buchu: effect of auxin and cutting type

Rooting of buchu: effect of auxin and cutting type

Abstract

Buchu (*Agathosma betulina* and *A. crenulata*) is grown commercially as an aromatic crop and is endemic to the Western Cape of South Africa. Poor rooting of cuttings has limited the development of superior clones. Under standard mistbed conditions, terminal, sub-terminal or basal stem cuttings taken from March to August and when not treated with an auxin, rooting percentages of between 20 and 25 are obtained. Rooting percentages increased to between 40 and 45 after treatment with 500-1000 ppm indolebutyric acid (IBA). Substituting IBA with naphthaleneacetic acid (NAA) did not improve rooting. There was a tendency for cuttings with fewer than four leaf pairs to give lower rooting percentages.

Introduction

In the past buchu has been solely harvested from the wild. At present about 50% of buchu oil and dried leaves sold are harvested from the wild (Wesgro, 2002). Factors limiting cultivation are lack of knowledge and the shortage of suitable plant material. At present buchu is propagated by means of seed. This resulted in great genetic variation in plants. Natural hybridization between the two commercially produced species has contributed to the variation of plant material and consequently, in oil quality and content of the plants.

Very little has been reported on the rooting of buchu. Von Wielligh (1913) reported that cuttings taken in autumn from wood of the previous year's growth could be successfully rooted. The cuttings were rooted in river sand, kept moist and covered with a bell glass or tumbler. After a few weeks the cuttings had rooted.

It has been repeatedly confirmed that applied auxins improve rooting of stem cuttings, and that the type of cutting and the number of leaves per cutting affect rooting

(Bean & Muroaka, 1974; Hambrick *et al.*, 1991; Garcia-Gomez *et al.*, 1994; Hartmann *et al.*, 1997).

This paper reports on the effect auxins and cutting type has on the rooting of buchu cuttings taken from March to August.

Materials and methods

Plant material

Sixteen mature *A. betulina x A. crenulata*, plants, selected from a commercial dry land plantation near Paarl (33°45'S; 18°58'E), Western Cape, South Africa, were used as a source for making cuttings. Plants were harvested in January 2000, after which no further pruning was done.

A second source of material for making cuttings consisted of *A. betulina* x *A. crenulata* hybrid plants grown from seed at the Fynbos Unit of the Agricultural Research Council, Elsenburg (33°55'S; 18°50'E), Western Cape, South Africa. Plants were grown in 5 litre containers, and irrigated and fertilised through a drip system. Plants were placed in a shade house with 60% shading. About one month before cuttings were taken, plants were cut back heavily and the shading increased to 80 percent. Cuttings were made when the new growth had elongated to 8cm.

Propagation system

The rooting medium consisted of a mixture of sand and peat (1:2), except for the NAA experiment where sand was used as the rooting medium. Trays of 34 x 34 x 9cm (49 plugs) were filled with the rooting medium and after the cuttings were planted, the trays were placed in a mist bed. The mist bed was bottom heated and the temperature of the rooting medium varied between 18-25°C. The cuttings were misted for 15 seconds every 15 minutes and the experiments terminated after 12 weeks. The percentage cuttings rooted was determined. A cutting was considered rooted if roots were seen on the outside of the plug when removed from the seedling tray.

Effect of indolebutyric acid (IBA) and naphthaleneacetic acid (NAA)

Terminal semi-softwood shoot cuttings were taken at four sampling dates (23 January, 22 March, 13 June, and 1 August 2001) from the Paarl source plants. The cuttings chosen were of similar thickness (approximately 2 mm) and length (6-8 cm). The bottom leaves were removed leaving four leaf pairs. The basal 5mm of the cuttings were dipped for 8 seconds in the IBA solutions (1 water: 1 ethyl alcohol).

Cuttings taken on 23 January and 22 March 2001 were treated with 500, 1000, 2000 and 4000 ppm IBA. For cuttings taken on 13 June, the 4000 ppm IBA treatment was replaced by a 250 ppm IBA treatment. An additional treatment of 125 ppm IBA was added to the treatments for the cuttings taken on 1 August. On June 15 cuttings were treated with 200, 400, 600, 800 or 1000 ppm NAA as described for IBA. Cuttings dipped in distilled water served as controls.

Cutting type

Soft, current year's growth was taken from shaded plants on 23 March 2001. Terminal, sub-terminal and basal cuttings, 8 cm long were made. Half of the leaves were stripped off the cutting leaving four leaf pairs. The basal 5mm of the cuttings were dipped into a 1000 ppm IBA solution for eight seconds.

Number of leaves

Semi-softwood cuttings 8 cm long were taken from the shaded plants on 18 July 2001. Leaves were removed from the basal part of the cuttings leaving two, three or four leaf pairs. Cuttings were all treated with 1000 ppm IBA as described in the previous experiment.

Statistical analysis and experimental design

A randomised complete block design was used with five blocks and 14 cuttings per treatment. Standard analysis of variance was performed on the data using the General

Linear Model procedure generated by the SAS® program (Statistical Analysis Systems Institute, 1996). Student's t-LSD was calculated at a 5% significance level to compare treatment means. All statistical analysis was done on logit- transformed data.

Results and Discussion

Auxins

Tables 1a, 1b, 1c and 1d show the percentage rooting achieved for cuttings taken in January, March, June and August respectively and treated with various concentrations of IBA or NAA (Table1e). Rooting in January was extremely low, 3 percent or less (Table 1a). Failure to effectively mist cuttings during this time of the year lead to the desiccation of the cuttings during the first week.

For cuttings made in March there was no significant difference in the rooting percentages for the different IBA treatments up to 2000 ppm. In this range IBA treated cuttings rooted consistently better than the control. Only 4 percent of cuttings treated with 4000 ppm IBA rooted (Table 1b). Cuttings treated with 4000 ppm IBA died back from the basal ends due to the toxicity at the high concentration of IBA. Cuttings treated in June with 500 ppm IBA had a rooting percentage of 42 percent (Table 1c), which is significantly better than the results achieved with higher concentrations IBA or in the control. Cuttings treated with 500 or 1000 ppm IBA in August resulted in the highest rooting percentage. This was significantly better than results achieved with lower concentrations, but was not significantly different from the control (Table 1d).

Cuttings treated with 1000 ppm NAA resulted in a significantly higher percentage (23 percent) of rooted cuttings than 600 or 800 ppm NAA but did not differ from other treatments (Table 1e). Better results may have resulted if higher concentrations were tested.

It appears that terminal cuttings of buchu taken from March to August and not treated with auxin have a rooting potential of 20-25 percent, whereas cuttings treated with IBA in the concentration range of 500 to 1000 ppm doubles the rooting potential. Although a 40

percent rooting is relatively low it is considered commercially viable. Due to the size of the cutting two cuttings per plug can be planted which will increase the effective use of the mist bed space. The rooting medium of sand was impractical, as roots tended to snap off the stem cutting when removed from the seedling tray.

Sample dates were chosen according to the growth pattern of buchu in the field. Flowering in buchu commences in winter (June) and continues until spring (late September) (Goldblatt and Maning, 2000). However, when buchu was harvested by pruning the shoots to just above where the shoots were harvested the previous years, in Jan-Feb. The shoot growth that develops after pruning, as a rule, does not flower during the period of June to September that follows the pruning treatment. Following this procedure ensures a good supply of cutting material available during the period March to August.

No callus developed during the rooting phase of buchu. In easy-to-root plants root formation is independent of callus formation (Hartmann, *et al.*, 1997). Callusing is, however, associated with difficult-to-root species such as *Pinus radiata* and *Hedera helix* (cited by Hartman, *et al.*, 1997). Roots formed from wounds induced by the removal of leaves when cuttings were made. Callusing is, however, not a prerequisite for rooting.

Leaf pairs

There was a general increase in rooting percentage with an increase in leaf pairs. The rooting percentage increased from 16 to 30 percent when leaf pairs per cutting were increased from two to four (Table 3). The positive effect of leaves on rooting of leafy cuttings is well documented (Bean and Muroaka, 1974; Reuveni and Raviv, 1981). It is therefore, recommended that buchu cuttings should consist of At least four leaf pairs.

Type of cutting

No significant difference in rooting of terminal, sub-terminal and basal cuttings were found. Rooting percentages varied from 43 to 45 percent (Table 4). In easily-to-root species, rooting is not affected by the position of the cutting. However, in difficult-to-root species terminal cuttings root most easily (Hartmann *et al.*, 1997). In buchu, it is an advantage that terminal, sub-terminal and basal cuttings can be used for rooting, especially when there is a shortage of material suitable for rooting.

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Tables

Table 1a.

Effect of IBA on the rooting of buchu (*A. crenulata* x *A. betulina)* terminal cuttings taken in January. Cuttings were taken from commercially dry land plantation. Cuttings were dipped in IBA for 8 seconds and placed in a sand: peat (1:2) mixture and evaluated after 12 weeks. Bottom heated (18-25°C) and open mist system (15 seconds every 15 minutes). Data represented are the mean percentages of 70 terminal cuttings per treatment.

Treatment	Percentage of rooted cuttings
Control	0 a
500 ppm IBA	3 a
1000 ppm IBA	1 a
2000 ppm IBA	0 a
4000 ppm IBA	0 a

* Values not followed by the same letter differ significantly at the 5% (LSD)

Table 1b.

Effect of IBA on the rooting of buchu (*A. crenulata* x *A. betulina)* terminal cuttings taken in March. Cuttings were taken from commercially dry land plantation. Cuttings were dipped in IBA for 8 seconds and placed in a sand: peat (1:2) mixture and evaluated after 12 weeks. Bottom heated (18-25°C) and open mist system (15 seconds every 15 minutes). Data represented are the mean percentages of 14 terminal cuttings per treatment.

Treatment	**Percentage of rooted cuttings**
Control	20 a
500 ppm IBA	43 a
1000 ppm IBA	27 a
2000 ppm IBA	41 a
4000 ppm IBA	4 b

* Values not followed by the same letter differ significantly at the 5% (LSD)

Table 1c.

Effect of IBA on the rooting of buchu (*A. crenulata* x *A. betulina)* terminal cuttings taken in June. Cuttings were taken from commercially dry land plantation. Cuttings were dipped in IBA for 8 seconds and placed in a sand: peat (1:2) mixture and evaluated after 12 weeks. Bottom heated (18-25°C) and open mist system (15 seconds every 15 minutes). Data represented are the mean percentages of 14 terminal cuttings per treatment.

Treatment	**Percentage of rooted cuttings**
Control	23 bc
250 ppm IBA	31 ab
500 ppm IBA	42 a
1000 ppm IBA	26 b
2000 ppm IBA	14 dc
4000 ppm IBA	13 d

* Values not followed by the same letter differ significantly at the 5% (LSD)

Table 1d.

Effect of IBA on the rooting of buchu (*A. crenulata* x *A. betulina)* terminal cuttings taken in August. Cuttings were taken from commercially dry land plantation. Dipped in IBA for 8 second and placed in a sand: peat (1:2) mixture and evaluated after 12 weeks. Bottom heated (18-25°C) and open mist system (15 seconds every 15 minutes). Data represented are the mean percentages of 14 terminal cuttings per treatment.

Treatment	**Percentage rooted cuttings**
Control	26 ba
125 ppm IBA	19 b
250 ppm IBA	7 c
500 ppm IBA	43 a
1000 ppm IBA	38 a
2000 ppm IBA	26 ba

* Values not followed by the same letter differ significantly at the 5% (LSD)

Table 1e.

Effect of NAA on the rooting of terminal cuttings of *A. crenulata* x *A. betulina* taken in June. Cuttings were taken from the commercially dry land plantation. Dipped in NAA for 8 seconds and placed in a sand: peat (1:2) mixture and evaluated after 12 weeks. Bottom heated (18-25°C) and open mist system (15 seconds every 15 minutes). Data represented are the means of 14 terminal cuttings per treatment expressed in percentage.

Treatment	**Percentage rooting of cuttings**
Control	12 ab
200 ppm NAA	15 ab
400 ppm NAA	13 ab
600 ppm NAA	4 b
800 ppm NAA	8 b
1000ppm NAA	23 a

* Values not followed by the same letter differ significantly at the 5% (LSD)

Table 2.

The influence of leaf pair numbers per cutting on the rooting of *A. crenulata* x *A. betulina.* Cuttings were dipped in 1000 ppm IBA for 8 seconds and placed in a sand: peat (1:2) mixture and evaluated after 12 weeks. Bottom heated (18-25°C) and open mist system (15 seconds every 15 minutes). Cuttings were taken from shaded mother plants grown in a tunnel. Data represented are the means of 70 terminal cuttings per treatment expressed as percentage.

Treatment	**Percentage rooting of cuttings**
2 leaf pairs	16 a
3 leaf pairs	23 a
4 leaf pairs (Control)	30 a

* Values not followed by the same letter differ significantly at the 5% (LSD)

Table 3.

The effect of cutting type on the rooting of *A. crenulata* x *A. betulina.* Cuttings were treated with 1000 ppm IBA and placed in a sand: peat (1:2) mixture and evaluated after 12 weeks. Bottom heated (18-25°C) and open mist system (15 seconds every 15 minutes). Cuttings were taken from shaded mother plants grown in a tunnel. Data represented are the means of 14 terminal cuttings per treatment expressed in percentage.

Treatment	**Percentage rooting of cuttings**
Terminal	43 a
Sub-terminal	39 a
Basal	45 a

* Values not followed by the same letter differ significantly at the 5% (LSD)

4. Paper II-

Rooting of buchu: effect of shading and carbohydrate levels

Rooting of buchu: effect of shading and carbohydrate levels

Abstract

Plants of *Agathosma betulina* x *A. crenulata* grown in Paarl and *A. betulina* grown in Piketberg were used as source plants for making cuttings. Paarl plants were shaded (80 percent shade) and Piketberg plants 60 or 80 percent shade from February to October 2002. Plants in full sun served as controls. Plants were pruned back initially in February and then samples were taken a month later in March. However, the plants were pruned back two months before the samples were taken in June, August and October at both locations. New shoots used as cuttings. Terminal cuttings for rooting and for carbohydrate analyses were collected at four dates (March, June, August and October). Cuttings were treated with 500 ppm indolebuteric acid (IBA) and placed in misting beds with bottom heating (18-25°C) for a period of three months.

Shading reduced rooting of cuttings from the Paarl plants and did not significantly influences the rooting of cuttings taken from Piketberg plants. Rooting percentage was highest in August (43%) for cuttings from sun grown plants in Paarl. No consistent relationship between dry mass or carbohydrate content of cuttings and rooting could be established.

Introduction

Buchu (*A. betulina* and *A. crenulata*) are aromatic crops, endemic to the Western Cape of South Africa. In the past, buchu has been solely harvested from the wild. At present about 50% of buchu oil and dried leaves sold are harvested from the wild (Wesgro, 2002).

At present, buchu is propagated by seed. The buchu industry has not taken advantage of selecting superior clones due to the difficulty in rooting buchu cuttings. In Paper 1, we reported that under standard mistbed conditions semi-softwood cuttings, made in March to

August and not treated with an auxin, have a rooting potential of ca 25 percent. Rooting percentages increased to between 40 and 45 percent when treated with IBA.

Procedures followed to increase the rooting of difficult-to-root items include blanching, etiolation and banding, and shading (Hartmann *et al.*, 1997). Softwood cuttings of buchu are very thin (2mm diameter) and etiolation and banding treatments were considered impractical and too expensive for this crop. It has been reported that cuttings taken from mother plants grown under shade root better (Klein, 1967; Johnson *et al.*, 1971; Delargy *et al.*, 1979; Read, 1987).

The exclusion of or reduction of light from shoots intended for propagation has shown to promote or be a contributing factor in adventitious rooting in a number of difficult-to-root plants; apple (Delargy *et al.*, 1979), *Pisum sativum* (Dolan, 1973), *Rhododendron spp.* (Read and Economou, 1987), *Phaseolus*, *Crassula argentea*, and *Rosa spp.* (Hartmann *et al.*, 1997). A relationship between adventitious rooting and carbohydrate status of cuttings have been expressed by Altman and Wareing, (1975); Breen and Muraka, (1973); Hansen, (1976) and Struve, (1981). It, however, remains unclear whether a relationship between carbohydrates and adventitious rooting exists.

In this paper, we report on the rooting and the content of non-structural carbohydrates of cuttings taken from mother plants of buchu grown in full sun or shade.

Methods and Materials

Plant material

Buchu plants, growing in two locations were selected as mother plants. Mature hybrid plants (*A. betulina* x *A. crenulata)* were selected from an established plantation near Paarl (33°45'S; 18°58'E), Western Cape, South Africa. The plants, ca 10 years old, were grown under dry land conditions. Plants were initially pruned in February and then two months before samples were taken in March, June, August and October at both locations.

At the second location near Piketberg (32°54'S; 18°45'E), Western Cape, South Africa, *A. betulina* plants were selected from a two-year-old plantation. Plants were irrigated through a drip system. Both areas are characterised by a Mediterranean-like climate of cool, wet winters and warm, dry summers.

Shading

Plants where covered with shade cloth mounted on steel frames from February to October, the last sampling date. Unshaded plants served as the control. At Paarl, 80 percent shade was applied and at Piketberg 60 or 80 percent. Sixteen plants were used at Paarl whilst, 30 plants were used at Piketberg.

Experimental layout

At Paarl, only four plants were shaded and cuttings were taken from one shaded and one unshaded plant at each sampling date. At Piketberg, 5 plants were used per treatment in a randomised complete block design. Unshaded plants served as control.

Rooting

Cuttings were made from shoots of current season's growth on 27 March, 11 June, 6 August and 8 October 2002 in Paarl and at Piketberg on 12 March, 6 June, 1 August and 1 October 2002. The cuttings chosen were of similar thickness (approximately 2 mm) and length (6-8 cm). The bottom leaves were removed leaving four leaf pairs. The basal 5mm of the cuttings were dipped for 8 seconds in the IBA solutions (v/v 50 water: 50 ethyl alcohol). The rooting medium consisted of a mixture of sand and peat (1:2). Trays of 34 x 34 x 9cm (49 plugs) were filled with the rooting medium and after the cuttings were planted, the trays were placed in a mist bed. The mist bed was bottom heated and the temperature of the rooting medium varied between 18-25°C. The cuttings were misted for 15 seconds every 15 minutes and the experiments terminated after 12 weeks. The percentages of rooted cuttings were determined. A cutting was considered rooted if roots were seen on the outside of the plug when removed from the seedling tray. Using 14 cuttings per treatment, treatments were replicated 5 times in a randomised complete block design.

Carbohydrate analysis

Cuttings were collected for carbohydrate analyses at the same time cuttings were collected for rooting. Cuttings had similar dimensions as described earlier for rooting.

Extraction

Half a gram of milled sample was placed into a 50mL centrifuge tube, 25mL of 5% acetic acid was added and then shaken for 14 h (Gerhardt shaker at 86 rpm). After the extraction period, samples were centrifuged at 3000-4000 g_n for 15 min. The supernatant was decanted into 100mL volumetric flasks. The supernatant contained the soluble sugars of the sample. The pellet containing the insoluble residue and the starch fraction were rinsed into 100mL volumetric flask with the specified acetate buffer (pH 4.8, 0.2M acetic acid plus 0.2M sodium acetate).

Starch

The volumetric flasks were half filled with the acetate buffer and gelatinized in a boiling steam bath for two hours. The suspension was cooled to 60°C. The starch fraction was hydrolysed to glucose with 100µL amyloglucosidase (200mg amyloglucosidase dissolved in 10ml acetate buffer) (Fluka Chemie, Buchs, Switzerland). Hydrolysis was performed in an incubator maintained at 55°C for 18 hours. The flasks were removed from the incubator and filled to 100mL mark with distilled water and then filtered through Whatman no. 2 (18,5cm) into sample bottles and stored at –20°C until further analysis on the Technicon Auto Analyzer.

Total sugars

One milliliter of Glycerol C (1000mL water and glycerol (1:1) + 200g activated charcoal) was added to the soluble sugar fraction. Samples were made up to the 100 mL with 5% acetic acid and filtered using Whatman no. 3 filter paper. Samples were bottled and stored at –20°C until used at a later date.

Analysis of reducing sugars and starch was done on a Sanplus Segmented Flow Analysis System (method numbers 551-965w/r issue 070798/MH and 356-001w/r issue 012998/MH97203066; Skalar, De Breda, The Netherlands).

Statistical analysis

Except for carbohydrate data all data was logit transformed. Standard analysis of variance was performed on the data using the General Linear Model procedure generated by the SAS® program (Statistical Analysis Systems Institute, 1996). Students' t-LSD was calculated at a 5% significance level to compare treatment means.

Results

Dry mass

Dry mass of terminal cuttings of the current season's growth from old plants in full sun in Paarl increased slowly from March to August and decreased rapidly thereafter (Figure 1a). In contrast, the dry mass of cuttings from shaded plants decreased consistently during the period of study. Young plants grown at Piketberg behaved differently (Figure 2a). The dry mass of terminal cuttings from both sun and shaded plants decreased from March to August and shade did not affect the dry mass. After August, dry mass of sun plants increased and continued to decrease in shaded plants.

Starch

The starch content of cuttings from shaded plants in Paarl was consistently lower than for sun plants (Figure 1b). Over the period of study, the starch content of cuttings from shaded plants varied from 1.7 to 4.3 mg as compared to 7 to 10mg for unshaded plants. For cuttings from plants grown at Piketberg the starch content of shaded plants were consistently below 5 mg except for 80 percent shade in August when double the value was registered (Figure 2b). This is possibly due to experimental error. Except for October when the starch content of the control cuttings were high (16mg), levels were low at other times.

Reducing Sugars

The sugar content of cuttings from shaded plants in Paarl decreased consistently from March to October (Figure 1c). During this period, the content dropped from 10 to 2mg. In cuttings of sun plants, the content was above 7mg except for the last date when 4 mg was recorded. The sugar content of cuttings from shaded plants in Piketberg was consistently lower than for cuttings from sun plants except for 80 percent shade in August (Figure 2b). In general, the sugar content decreased as the season advanced.

Rooting

The highest rooting percentage achieved with cuttings from the Paarl plants was for cuttings taken in August from non-shaded plants, which was 15 percent higher than the 25 percent achieved for shaded plants (Figure 3). Cuttings taken at any other time, whether from shaded or non-shaded plants rooted poorly and varied from 2 to 20 percent. Cuttings from sun plants at Piketberg rooted poorly, irrespective of the date of collection (Figure 4). Rooting dropped from 15 percent in March to zero in October. Thirty three percent of the cuttings taken in June from plants grown in 80 percent shade rooted. This is 13 percent better than the second highest percentage obtained for cuttings taken in August from plants under 60 percent shade.

Discussion

In Paper 1, we reported that between 40 and 45 percent of buchu cuttings taken between March and August rooted when treated with IBA and placed in standard mist bed conditions. Buchu flowers from June to September on shoots that grew the previous summer. The intra-plant factors as well as the environmental factors that control flowering in buchu is unknown. In woody perennials, such as Citrus (Furr *et al.*, 1947, Krajewski and Rabe, 1995) immature shoots fail to flower in spring because they are non-responsive to inductive conditions of winter, low temperatures. Hard pruning of buchu plants in summer produced in most cases non-flowering shoots suitable for making cuttings in March to October. A second approach to prevent or reduce flowering is to grow plants

under low light intensities. Flowering in many, if not most, plants are inhibited by low light. Since the seedling plant of buchu grown in Paarl flowered profusely even after being pruned back hard and covered with 80 percent shade is indicative of the genetic variability in precocity to flower. This explains the missing data for the rooting experiment with cuttings taken in June from Paarl.

In Paper 1, we also reported that the rooting potential of buchu cuttings is between 40 and 45 percent. The rooting percentages varied for cuttings taken from sun plants from the two areas as well as for the different sampling dates. The reason for this variability is poorly understood. Comparable variable rooting results were also reported in Paper 1 on work done the previous year. There is no obvious reason why cuttings from Paarl taken in August rooted to its apparent potential but not when taken at other times or from the Piketberg source. Neither is there a clear relationship between the rooting response and the dry mass of cuttings, nor starch or sugar content. For example, the dry mass and starch and sugar content of cuttings from sun plants in Paarl were comparable during March and August, yet only the cuttings taken in August gave a fair rooting percentage. The effect of shading mother plants on rooting of cuttings is also not consistent. Shading Paarl plants generally resulted in poorer rooting. Although this can be ascribed to the shade-induced decrease in dry mass and starch and sugar content of the cuttings, this argument does not hold true for the Piketberg cuttings. Cuttings from shaded plants in some cases rooted better than cutting from sun plants, yet differences in dry mass and starch and sugar content between sun and shaded plants were not that apparent.

Considering the available information, the following procedure is recommended for rooting of buchu cuttings under standard mist bed conditions. Cuttings should preferably be taken from mother plants prepared specifically for this purpose. Mother plants should be established in suitable sized containers. During January, the plants should be pruned back hard to encourage new shoots. When new shoots are ca 30 cm long both terminal and sub-terminal cuttings can be made for rooting. Continuous pruning of the mother plants to supply cuttings for rooting will reduce the incidence of shoots developing flowers. It is also recommended that mother plants be kept under 30-40 percent shade to further reduce the likelihood of flower formation. Cuttings should be treated with 500-1000 ppm IBA.

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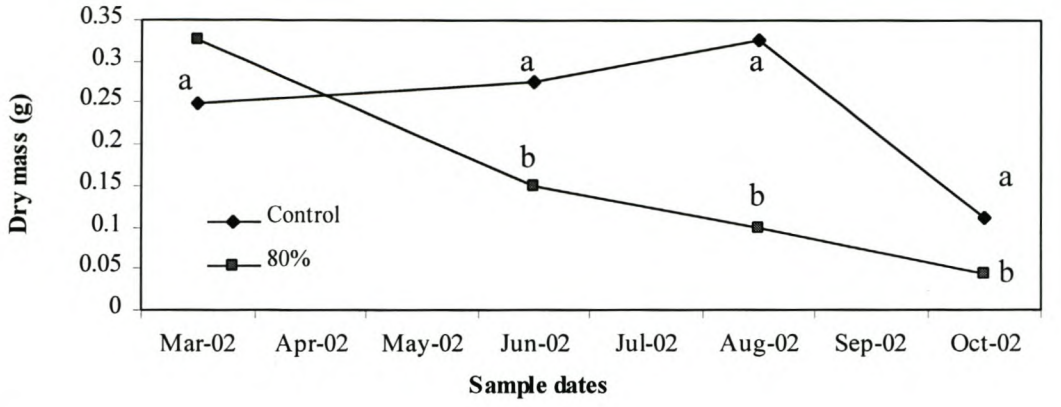

Fig. 1a

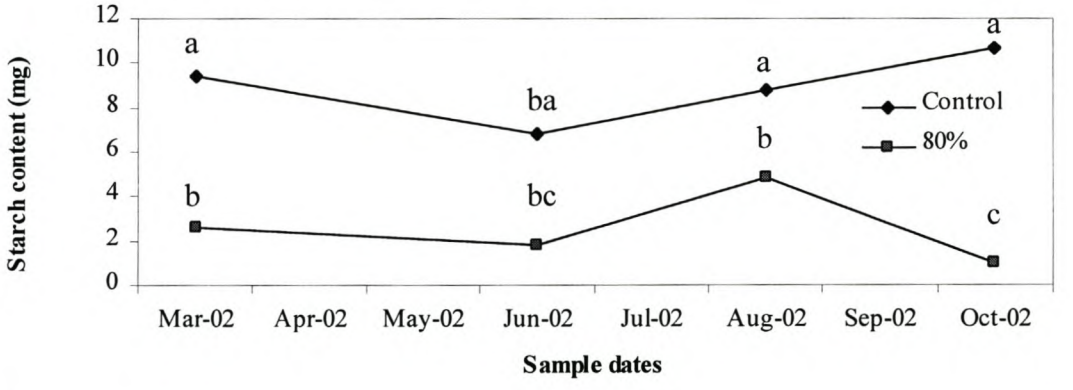

Fig. 1b

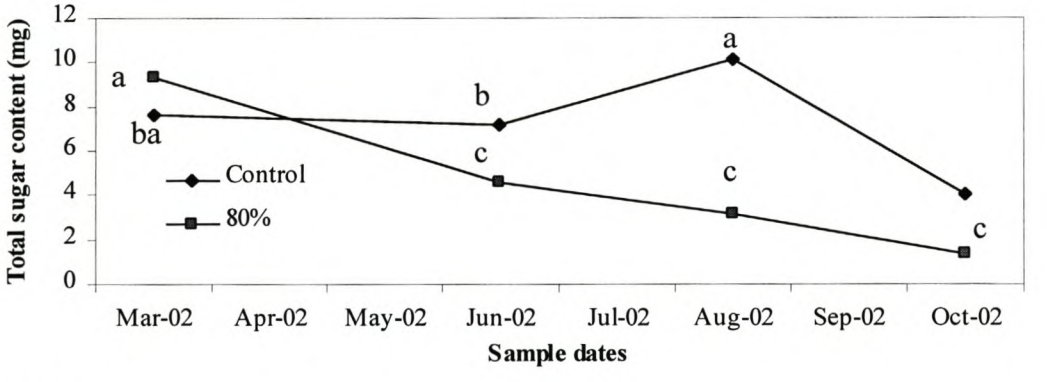

Fig. 1 c

Fig. 1 a, b & c.

Effect of shading (0, 80%) on dry mass, starch and total sugar content per terminal cuttings of *A. betulina* x *A. crenulata* (Paarl) at four stages during the growth cycle. Total sugar includes the reducing and non-reducing sugars. Data presented are the average of 70 cuttings. Different letters indicate significant differences at 5% within individual shading times.

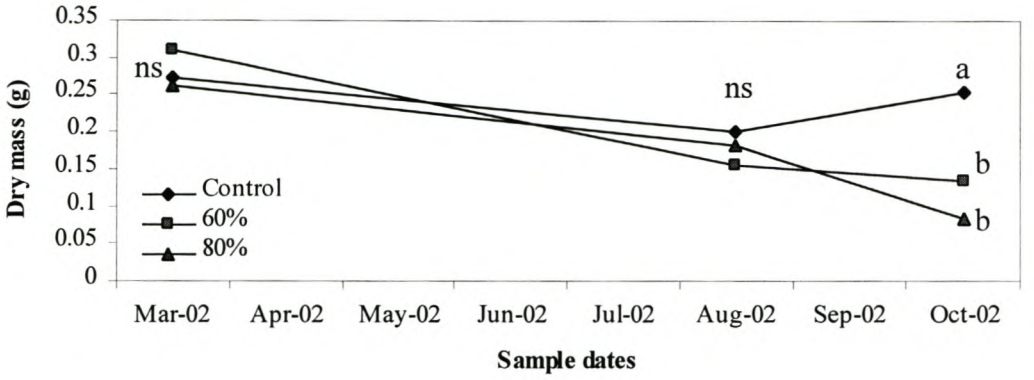

Fig. 2a.

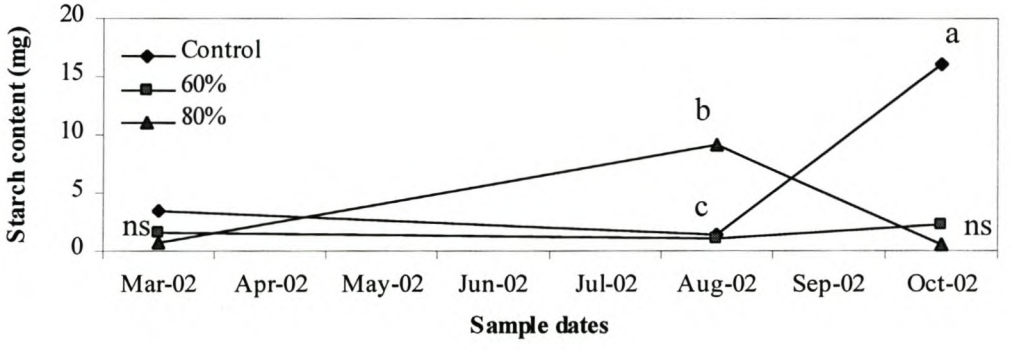

Fig. 2b.

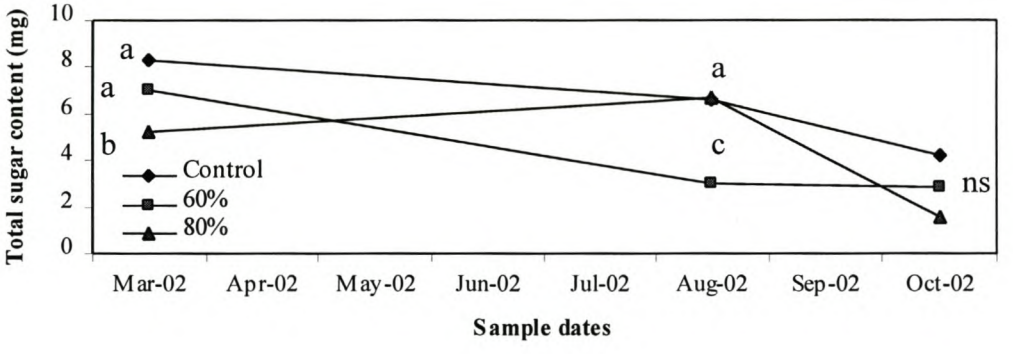

Fig. 2c.

Fig. 2 a, b & c.

Effect of shading (0, 60 & 80%) on dry mass, starch and total sugar content per terminal cuttings of *A. betulina* (Piketberg) at three stages during the growth cycle. Total sugar includes the reducing and non-reducing sugars. Data presented are the average of 70 cuttings. Different letters indicate significant differences at 5% within individual shading times.

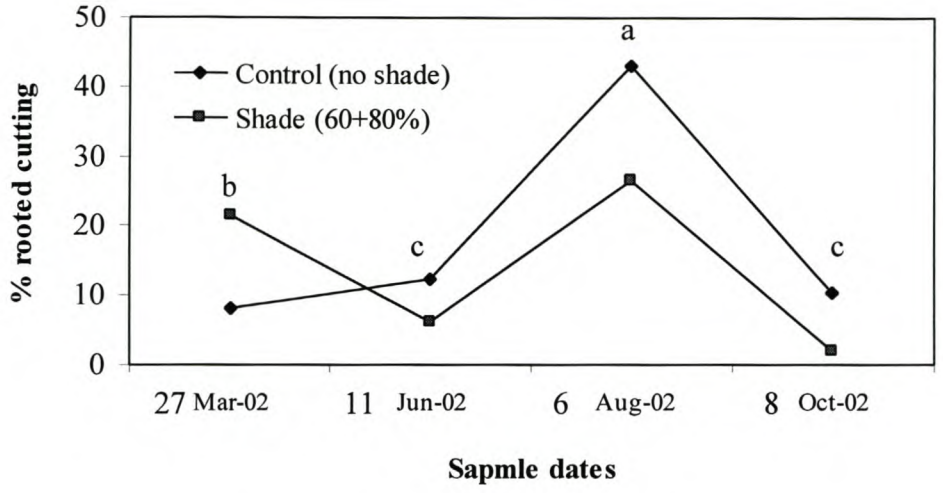

Fig. 3:
The mean percentage of rooted cuttings of *A. betulina* x *A. crenulata* (Paarl) under shade taken at four stages during the growth pattern. Cuttings were treated 500 ppm IBA and placed in a sand: peat (1:2) mixture and evaluated after 12 weeks. Bottom heated (18-25°C) and open mist system (15 seconds every 15 minutes). Data presented are the average of 70 cuttings. Means with different letters show significant interaction between time and treatment at 5%.

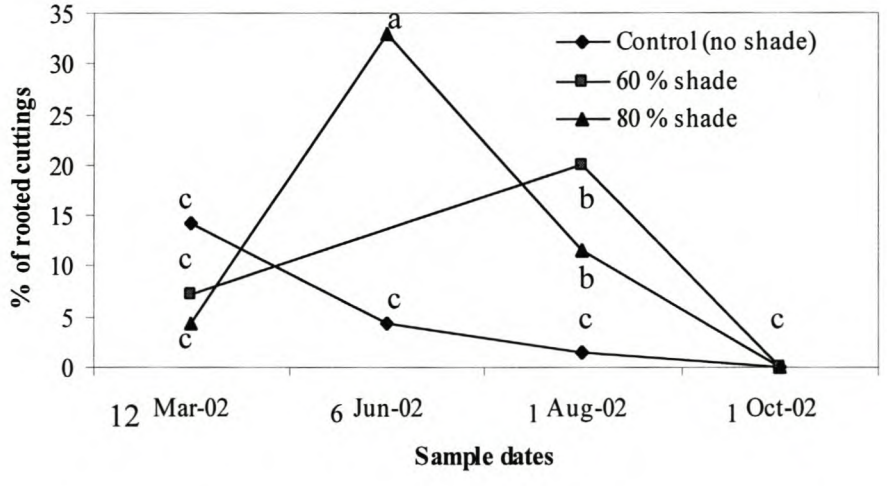

Fig. 4.
The mean percentage of rooted cuttings of *A. betulina* (Piketberg) under shade taken at four stages during the growth pattern. Cuttings were treated with 500 ppm IBA and placed in a sand: peat (1:2) mixture and evaluated after 12 weeks. Bottom heated (18-25°C) and open mist system (15 seconds every 15 minutes). Data presented are the mean of 70 cuttings. No significant difference at 5% was seen in shading or at sampling dates.

5. Paper III-

Rooting of buchu: rooting co-factors

Rooting of buchu: rooting co-factors

Abstract

Terminal current years' growth, taken from *Agathosma crenulata* x *A. betulina* (hybrid) softwood cuttings collected in January 2002 were extracted with methanol and fractioned by thin layer chromatography (Silica gel) in isopropanol: acetic acid: water (4:1:1 v/v). The chromatographs were divided in ten fractions and were bioassayed for rooting co-factors with the mung bean rooting test. Extracts from buchu cuttings showed significant activity at the Rf values of co-factor 3. However, co-factors 1, 2 and 4 do not seem to be present in significant quantities, but instead co-factors with Rf values different from previous reported values are present in significant quantities. No inhibition was found in buchu In fact all Rf values stimulated rooting.

Introduction

Buchu (*A. betulina* x *A. crenulata*), an aromatic crop, endemic to the Western Cape of South Africa was solely harvested from the wild in the past. At present about 50% of buchu oil and dried leaves sold are harvested from the wild (Wesgro, 2002). Besides the favourable medicinal properties of buchu, a great economical importance lies in the extraction of its foliar oils for export. Buchu oil is used to enhance and fix the inherent flavour of mango, tropical and berry flavourants (Masciano *et al.*, 1994). Buchu oil is also used in perfumes, cosmetics and soaps (Roberts, 1997).

Considerable difficulties have been expressed in the rooting ability of buchu cuttings [4](pers. comm., Blomerus). The ontogenetic age of the plant source is critical in the success

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[4] Blomerus., L., 2003. Agricultural Research Council. Fynbos Unit. Private Bag X1, Elsenburg, 7607. South Africa.
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of rooting (Heuser, 1976). Generally plants in the juvenile phase root more easily. Hess, (1967) found juvenile ivy to root more easily than that of mature ivy (*Hedera helix* L).

Although auxin can increase the rooting potential of a large number of plants, there still exist difficult-to-root forms, which respond very little to auxin treatments (Hartmann *et al*, 1997).

The inability to achieve satisfactory rooting with known root-promoting compounds stimulated a search for other naturally-occurring substances. Many juvenile and easy-to-root plants contain substances, called rooting co-factors, which are capable of stimulating rooting together with auxin. Endogenous rooting factors other than auxin, are thought to control rooting, and are produced in leaves and buds or both. Such factors are said to be occuring in large amounts in easy-to-root cuttings, but present in smaller quantities, if at all, in difficult-to-root types . (Hess, 1962b; Fadl and Hartmann, 1966; Hackett, 1970). Juvenile ivy has a higher rooting capacity, due to the presence of 'rooting co-factors' (Hess, 1967; Heuser, 1976). Co-factors were considered to be endogenous substances capable of acting synergistically with IAA in the rooting of cuttings from seedlings of the mung bean (Hess, 1957 cited in Girouard and Hess, 1966).

Plants can be classified in respect to there rooting ability:

1. Rapid-rooting plants: these plants contain all the necessary substances required for rooting (auxin, co-factors). These plants therefore root under favourable environments.
2. Auxin-requiring plants, which have all the internal co-factors needed for rooting but lack auxin.
3. Co-factor deficient: they lack one or more co-factors and rooting doesn't occur by just adding auxin.

Adventitious root production by cuttings of mung bean seedlings has been extensively used to detect and measure substances, which promote rooting (Girouard and Hess, 1966; Gorter, 1969; Blazich and Heuser, 1979). The mung bean cuttings were chosen because of their insensitivity to indoleacetic acid (IAA) unless one or more of a group of substances that are generally called rooting co-factors are present (Jackson and Haney, 1969). The test provides a method to detect substances other than auxin, thought to

be necessary for root initiation and demonstrate a deficiency in some difficult-to-root cuttings of other species.

The basic methodology of Hess (1962a) was used to determine the presence of co-factors in buchu. The cuttings were tested for rooting responses to the rooting co-factor substances extracted from *A. betulina* x *A. crenulata* tissue and indolebutyric acid (IBA).

Method and Materials

Plant material

Mature *A. betulina x A. crenulata*, hybrid plants, selected from an established commercial dry land plantation near, Paarl (33°45'S; 18°58'E), Western Cape, South Africa.

The exact age of the plants were unknown but were estimated to be older than ten years. Fourteen cuttings, 8cm long, were used in each replication. The material was frozen at –20°C, freezedried and milled into a fine powder. Samples were taken for analysis, from each treatment and replicated 3 times.

Extraction of rooting co-factors and chromatography

The co-factors were extracted from 0,5 g of leaf and stem tissue with 25ml of 70% methanol (Jackson *and Harney,* 1970). The extract was fractioned using thin layer chromatography (TLC) (Silica gel RP-18, F254S). The chromatographs were equilibrated for 1 hour. Three milliliters of the extract was streaked on the plate and placed in the TLC tank for 6-10 hours and developed at 22°C by ascending chromatography in isopropanol: acetic acid: water (4:1:1 v/v). Plates were removed when the front was two centimeters from the top of the plate and dried.

Chromatograms were divided into 10 sections and residue on each section was separately scrapped off the plates. Ten milliliters of methanol (70%) was added to the powder and centrifuged (Sorvall RC Refrigerated Super Speed Centrifuge 12 min 0-15°C;

12350g_n). Five milliliters of the supernatant was extracted from the top fraction. The incubation medium consisted of 1 ml of the extract and 4 ml of 10 mg/l IBA.

The root-promoting activity of the sections of the chromatography plates was determined by a mung bean bioassay.

Mung bean bioassay

The material was prepared by using the basic methodology of Hess (1962b) and Yopp *et al.* (1986). *Phaseolus aureus* (mung bean) was used. Seeds were germinated by running tap water over the seeds for 7 hours.

The germinated seeds were planted 0.5 cm deep in moistened sand: peat mixture (1:2) in plastic trays. Trays were placed in a growth chamber (climate cabinet economic deluxe EC 01-089) for 7 days. The temperature was maintained at 22-25°C with a relative humidity of between 70-75 percent. The seedlings were under a constant light source of ($200\mu E.m^{-2}.s^{-1}$).

Seedlings were cut with a sharp razor 3cm below the cotyledon. Uniform cuttings, consisting of a 3cm hypocotyl, epicotyl, the unifoliate leaves and the trifoliate leaf bud were chosen. The cotyledons were carefully removed.

An incubation medium consisting of 1ml of the extract and 4 ml 10mg L idolebutyric acid was made. Ten cuttings were placed in each vial. Basal ends of cuttings were placed in the incubation medium for 48 hours and then placed in larger vials containing 10 ml of double distilled water. The water absorbed by the cuttings was replenished every 24 hours for a 4 day period. Three replicates were used per treatment.

Results and Discussion

On average five roots per cutting were induced by IBA on its own (Fig. 1). The addition of extracts from buchu cuttings to the rooting solution increased the number of roots per mung been explants, irrespective of the Rf position of the fraction on the chromatogram. Significant more roots formed per explant when extracts with Rf values of 0.22-0.33; 0.53-0.62; 0.71-0.81 and 0.9-1.0 were added to the rooting medium.

Hess (1962b) established the Rf values for co-factors in work done on *Hedera helix* L. and red flowering *Hibisccus rosa-sinensis* L (Girouard and Hess, 1966). Four co-factors were found to be active. The R_f values for the cofactor 1, 2, 3, and 4 were between 0-0.013, 0.33-0.53, 0.6-0.73 and 0.8-0.93, respectively. Co-factors one to four have also been found in easy-to-root chrysanthemum, in large amounts (Heuser and Hess, 1966). There is some agreement between our results and those of earlier reports (Hess, 1962b) but also discrepancies. It appears that co-factor 3 is also present in buchu. However, co-factors 1, 2 and 4 does not appear to be present in significant quantities, but instead co-factors with Rf values different from previous reported values are present in significant quantities. The buchu extracted also differs in respect of inhibitors of rooting when compared to previous reports. In previous reports (Hess, 1962b), fractions of the plant extract located in specific Rf positions on the chromatograph inhibited rooting. No such inhibition of rooting was found for buchu. In fact all Rf positions stimulated rooting.

It appears therefore that lack of co-factors is not the cause for poor rooting of buchu. Since rooting of buchu cuttings is promoted by auxin (Paper 1), buchu falls in the category of plants that are expected to root easily. Poor rooting of buchu is related to the long period it takes for the first roots to appear. The diameter of buchu cuttings varies between 1 and 2mm and has ca 4 needle-like leaves. This makes cuttings very vulnerable to stress as would occur if mist bed conditions are unfavourable, even for short periods of time as would occur during a heat wave, or interruption of the mist system caused by a power failure. In summary, therefore the successful rooting of buchu will depend on the proper preparation of the mother plants as a source of suitable cuttings, treatment of cuttings with IBA and meticulous care of the mist bed conditions during the rooting phase.

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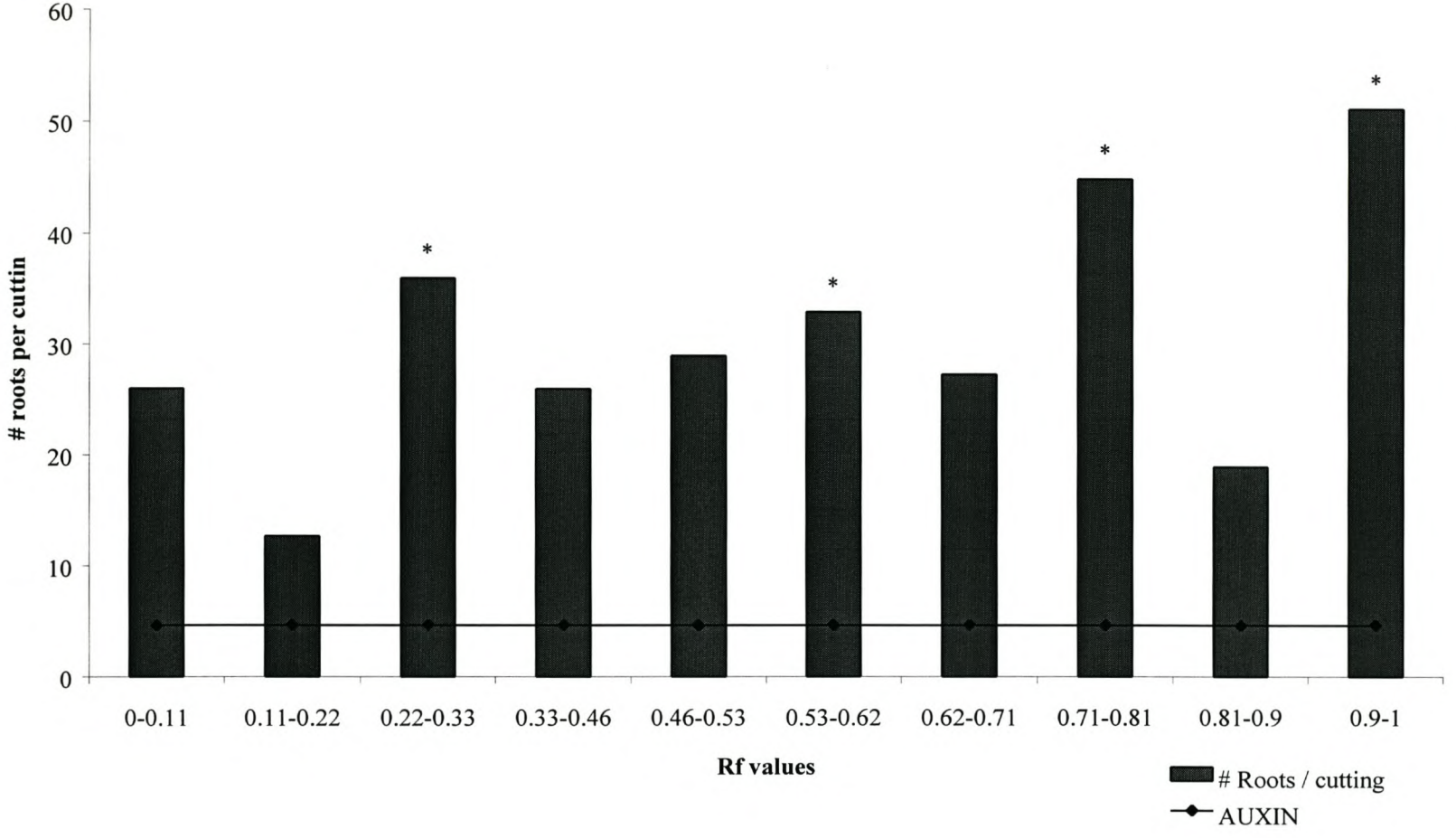

Fig. 1. Mung bean bioassay for rooting of co-factors in buchu. Number of roots per explant for different fractions of buchu extract. Chromatograph fractioned methanol extracts of terminal current season's growth of A. *crenulata* x *A. betulina* when tested with mung bean bioassay. Chromatograph developed isopropanol: acetic acid: water (4:1:1 v/v). * Differs significantly from the auxin control.

6. General discussion and conclusion

The propagation of buchu by means of cuttings is commercially feasible and is shown in Paper 1 and 2. The rooting percentages improved from 20-25% without IBA to 40-45% when 500-100 ppm IBA was applied. A rooting success of 40% is commercially plausible. No rooting inhibiting factors were found in buchu in Paper 3. The results of the three papers suggest that buchu is a relatively easy-to-root plant. Since buchu cuttings are inherently slow to root poor rooting results are related more to the ability of the cutting to survive for long enough for rooting to take place. The dry mass and available carbohydrates of softwood cuttings are low. Further more the young softwood cuttings are very susceptible to drying out or stress caused by low water potentials in the cutting may inhibit rooting. We conclude that for successful rooting of buchu softwood cuttings meticulous attention should be paid to mist-bed conditions to prevent water stress of the cuttings. Fogging systems should possibly be considered for rooting softwood cuttings of buchu.

Successful rooting of buchu softwood cutting is also dependent on the quality of the cutting. It is suggested that mother plants for the production of cuttings should be established. Mother plants could either be established in containers or in the open ground in either case under ca 30% shade. Well in advance of the time when cutting are needed mother plants should be pruned to force out new shoot suitable for cuttings.

The successful rooting of buchu cutting now necessitate that the next steps in the upgrading of the buchu plant materials are implemented. These would include the selection of superior seedling mother plants based on yield, oil content and oil quality and ease to root cuttings. Plants established in the open from rooted cuttings of the selections should then be studied in more detail for their commercial value.