Vegetative propagation of *Pappea capensis* Eckl. & Zeyh. (Jacket plum) by means of stem cuttings and air layers

by

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DECLARATION

I, the undersigned hereby declare that this dissertation, submitted for the degree of MSc (Agric.) Horticulture at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at another University.

Signature……

Date……

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“The credit belongs to the man who is actually in the arena; whose face is marred by dust and sweat and blood”

Theodore Roosevelt

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ABSTRACT

Jacket plum [*Pappea capensis* (Eckl. & Zeyh)] belongs to the Sapindaceae or Litchi family. It is well adapted to different climatic conditions. It has been used for medicinal purposes for both animals and human beings. Due to the richness of seeds in oil, it has great potential to be selected for production of biodiesel in South Africa. Suitable vegetative propagation methods for *Pappea capensis* trees have not yet been investigated and sexual propagation does not produce true-to-type plants, which take many years to bear fruits. Therefore, research was carried out to identify alternative methods for vegetative propagation of *Pappea capensis* which could be used for rapid multiplication.

Several vegetative propagation experiments were carried out with stem cuttings and air layers. Stem cuttings were collected from two mature *Pappea capensis* trees grown at the Experimental Farm of the University of Pretoria in the spring and autumn seasons. The cuttings were placed for rooting on the mist bed for rooting with and without Seradix® No. 2 [active ingredient, indolebutyric acid (IBA)] treatment. Other experiments followed in which the branches were girdled before making stem cuttings to improve the level of any carbohydrates or available carbohydrates. Trials to investigate the rooting potential of *Pappea capensis* coppices, using different stem lengths, were also conducted. Rooting of *Pappea capensis* stem cuttings was unsuccessful.

Air layers were made on the same trees where cuttings were collected. The trials were conducted in the spring and autumn seasons from 2006, 2007 and 2008. Some air layers were treated with Seradix® No. 2 and others were not treated with the auxin. High rooting percentages (100% in spring, 60% in autumn) were achieved with untreated air layers of Tree No. 1 and Tree No. 2 (80% in spring, 40% in autumn). Regardless of season, IBA and tree treatments, rooting was successful when the air layering method was used.

Due to inconsistency in rooting from both vegetative methods, total phenolic compounds were extracted. The Folin-Ciaocalteau reagents method was used to extract phenolic compounds and the results were detected with Elisa reader instrument. The stem cuttings and air layers were further analysed for carbohydrates (starch and soluble sugars) with σ-toluidine reagent and ethanol and read with Spectrophotometer and high performance liquid chromatography (HPLC). Higher levels of total phenol compounds were observed from callused (27.13 mg/g) and non rooted untreated (26.41 mg/g) stem cuttings from Tree No. 2, compared to IBA treated stem cuttings (19.90 mg/g) of callused and non rooted IBA treated stem cuttings (20.25 mg/g) of Tree No. 2. High total phenols (34.55 mg/g) in untreated air layers were also found in callused air layers of Tree No. 2 and lower amounts (22.85 mg/g) in treated air layers of the same tree. No soluble sugars were detected in stem cuttings or air layers with HPLC. Regarding starch, higher amounts were observed in stem cuttings of Tree No. 1 (18.45 mg/g) of the control and Tree No. 2 (19.82 mg/g) of IBA treated cuttings. Most of the air layers made on Tree No. 1 had higher percentages of starch, with the exception of the callused (7.41 mg/g) air layers of the control. Tree No. 2 air layers had very low amounts of starch when compared with those of Tree No. 1.

The variation in rooting potential of stem cuttings and air layers led to the consideration of tree gender as a factor influencing success rates, where inflorescences were collected from the two *Pappea capensis* trees for two years (2007 and 2008). The microscopic investigations showed that *Pappea capensis*

trees (Tree No. 1 and Tree No. 2) were monoecious, however, Tree No. 2 switched from monoecious to male by producing only male flowers.

Based on the results of the above investigations, air layering in the spring season can be used as a (alternative) vegetative propagation method for *Pappea capensis* tree, but on specifically monoecious tree to obtain higher rooting percentage. However, these are preliminary trials which require further investigation.

Keywords: *Pappea capensis,* stem cuttings, air layering, season, total phenols, soluble carbohydrates and tree gender

RESEARCH OUTPUT

The following paper was orally presented:

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GENERAL INTRODUCTION

Pappea capensis (Eckl. & Zeyh.) belongs to the *Sapindaceae* or Litchi family (Fig. 1.1). It is commonly known as Jacket plum. The tree was named after the colonial botanist, Dr. Ludwig Pappe (Fanie & Venter, 1996). Different common names are used at different places where it grows in Southern Africa such as Doppruim, Mongatane, Umqhokwane, Indaba tree, Bushveld cherry, Mopennweeng, Mopsinyugane and Liletsa (Jooste, 2003).

It is an attractive tree that has dense light grey and smooth branches (Fanie & Venter, 1996). Its leaves are leathery and crowded at the tips of the branches. *Pappea capensis* is widely distributed in southern Africa, extending northwards into eastern tropical Africa as far as Ethiopia (Hankey, 2004). It is well adapted to different climatic conditions and it is then cultivated in homesteads as it is found in England and in Zimbabwe (Palmer & Pitman, 1961). With enough supply of moisture, it becomes evergreen and in arid areas, the tree becomes deciduous. This species is drought tolerant and mature trees withstand moderate frost.

Jacket plum is a multi-purpose tree because it is used for many things; it produces edible jelly-like fruits with an orange-red flesh and sour taste. According to van Wyk & Gericke (2000), most of the edible indigenous fruits, when analysed, were found to have high contents of vitamin C. These fruits are made into jelly, vinegar and alcoholic beverages. Apart from that, leaves, roots and bark are used for medicinal purposes (Fanie & Venter, 1996). Its magnificent seeds are rich in oil, which is usually used to make soap, to oil guns and its oil is used as medicine, for example to treat ringworms.

Having seed rich in oil, Jacket plum could be selected for the potential production of biodiesel in South Africa. Furthermore, this tree can be planted in marginal land because it can withstand the harsh climatic conditions caused by erratic

rainfall. Such a biodiesel project would create job opportunities for rural communities and the problems of poverty reduction as well as unemployment would be addressed. However, Jacket plum planting material is going to be in high demand if a project for biodiesel production is established in South Africa and suitable propagation methods should then be identified.

At present, the tree is only propagated sexually with seeds (Van der Schiff, 2006) and its slow germination rate can be improved by removing the seed testa (Mng'omba & du Toit, 2005). But seeds do not produce true-to-type seedlings, which resemble their parents. Propagation by stem cuttings, grafting and air layering, however, would facilitate cloning of desirable genotypes, possibly leading to named cultivars (Macdonald, 1986; Hartmann, Kester & Davies, 1990). Therefore, vegetative propagation of *Pappea capensis* can be the best solution to maintain genetic uniformity and encourage a shorter cycle of crop production. The success of the technique requires proper balanced phytochemicals of the plant, temperature, rainfall, humidity, nature of media and light that collectively decide the status of regeneration of roots in cuttings (Eganathan, Rao & Anand, 1999).

Nevertheless, there is no published scientific information available on the conventional vegetative propagation of Jacket plum. Therefore, the study is envisaged to find out the effective method of vegetative propagation of the tree. Information regarding vegetative propagation of this tree could benefit the producers, especially those aiming for producing *Pappea capensis* planting materials.

Aim of the study

Investigation of the vegetative propagation of *Pappea capensis* (Jacket plum) includes production of plants by stem cuttings and air layering and studying the effects of phenolic compounds and carbohydrates on rooting. The aims of the study were to:

- Generate protocols for vegetative propagation of *Pappea capensis*
- Determine rooting potential of stem cuttings
- Determine the feasibility of:
    - air layering (marcottage) as an alternative vegetative propagation technique
- Investigate seasonal effects
- Investigate application of auxin to improve rooting
- Observe the flowering of two *Pappea capensis* trees at the Experimental Farm, University of Pretoria in relation to vegetative propagation
- Quantify phenolic compounds and carbohydrates and investigate their possible role in the rooting of stem cuttings and air layers

CHAPTER 1

LITERATURE REVIEW

1.1 Vegetative propagation

Vegetative or asexual propagation can be defined as the production of a plant so that the offspring will contain the exact characteristics of the mother plant in genotype as well as health status (Macdonald, 1986; Hartmann, Kester & Davies, 1990). Vegetative propagation is an important tool for crop improvement. Its aim is the formation of adventitious roots and/or shoots on stems, leaves or roots for successful plant regeneration. According to Hartmann *et al.* (1990) vegetative propagation is possible because the living cells contain genetic information in their nuclei necessary to reproduce the entire plant. In asexual reproduction, growers have the ability to obtain a high degree of crop uniformity and a high quality product is achieved. However, vegetative propagation can sometimes result in intraclonal variation which is comparable to variation within a sexually propagated population (Swamy, Puri & Singh, 2001).

There are several reasons for using vegetative propagation, including the convenience and ease of propagation, selection and maintenance of clones, combination of more than one genotype into a single plant, as well as a shortened time to reproductive maturity (Hartmann *et al.*, 1990). For most crops, vegetative propagation is not a natural occurrence, therefore special and expensive production structures or facilities such as greenhouses and mist propagation chambers may be needed. This could result in expensive production of plant material when compared with sexual propagation. Furthermore, in clonal propagation superior plants are used and when reproducing asexually, it fixes genetic variation in contrast to a sequence of generations required for seedling populations. Sometimes different cultivars are combined into a composite plant as scion, rootstock and interstock, each part providing a special characteristic.

Hartmann *et al*. (1990) reported that vegetatively propagated crops flower earlier than seedling plants because they grow faster than plants produced from seeds. Vegetative propagation provides the best opportunity to multiply valuable trees for cultivation (Leakey & Simon, 1997).

In vegetative propagation from stem cutting and air layers, adventitious root formation is a prerequisite to successful propagation. There are two types of adventitious roots; namely preformed and wound-induced roots. Lovell & White (1986) defined preformed or lateral roots as roots which lie dormant until the stems are made into cuttings and placed under environmental conditions favourable for further development and the result is emergence of the root primordia as adventitious roots. Wound-induced roots are developed only after the cuttings are made in response to wounding during the preparation of the cutting (Lovell & White, 1986). In fact, wounded cuttings respond to protect themselves from infection and desiccation by production of an irregular mass of parenchyma cells or callus at the wound and then roots emerge through the callus.

Vegetative propagation of plants can be done by using cuttings, layering, grafting, budding and micro-propagation. The literature review will only be on stem cuttings, and air layering, and how exogenously applied auxin (IBA), phenolic compounds and soluble carbohydrates affect production of adventitious roots.

1.1 Vegetative propagation by stem cuttings

Stem cutting propagation is an ancient technique and it has been traced as far back as ancient China. Vegetative propagation by stem cuttings has the ability to produce a large number of young plants from a single parent plant, thus it is a useful technique in conservation of endangered plants (Macdonald, 1986) and rapid propagation of new cultivars.

A stem cutting is a vegetative part of the plant obtained from the stem, which is detached from the mother plant and is capable of regenerating itself into a composite plant. Cuttings are classified according to the part of the plant from which they are obtained such as stems, modified stems (rhizomes, tubers, corms and bulbs), leaves and roots (Hartmann *et al*., 1990). According to Hartmann *et al*. (1990) stem cuttings can be grouped into hardwood, semi-hardwood, softwood, and herbaceous. In propagation by stem cuttings, rooting is influenced by many factors including type of wood, the stage of growth when cuttings are made, the time of year when cuttings are taken, rooting medium, rooting auxin and physical factors (Macdonald, 1986; Hartmann *et al*., 1990; Wilson, 1993).

1.1.1.1 Type of wood

Stem cuttings are grouped into hardwood, semi hardwood softwood and herbaceous depending on the nature of wood used (Hartmann *et al*., 1990), based on the stage of stem growth development (Macdonald, 1986). Rooting ability may vary between cuttings obtained from different parts of the same plant, or at different times of the year, especially in woody species and this is related with stem structure and chemical composition in the stem (Macdonald, 1986; Leakey & Mohammed, 1985; Hartmann *et al*., 1990). In difficult-to-root species, when the stem matures and gets older a continuous sclerenchyma ring may grow between the phloem and cortex outside the point of origin of adventitious roots, and may hinder free root growth, whereas in easy-to-root species, fewer sclerenchyma cells or layers are formed (Hartmann, Kester, Davies & Genever, 1997).

Hardwood cuttings

Hardwood cuttings are made from mature *dormant* hardwood before new shoots emerge in the spring (Hartmann *et al*., 1990). Vegetative propagation from dormant season hardwood is found to be an efficient and increasingly used practice for a number of deciduous tree species (Robinson & Raffa, 1996). Japanese Marubakaido (JM) series rootstock clones are, for example, propagated easily by hardwood cuttings (Soejima, Bessho, Tsuchiya, Komori, Abe & Kotoda, 1998). Also, Nicotra & Pellegrini (1989) noticed that hardwood cuttings of nectarine varieties have clearly higher rooting ability than those of peach varieties. Nicotra & Pellegrini (1989) further explained that the easy propagation characteristic is a hereditary thing which is regulated by many genes.

Many studies indicated that older (mature), lignified stem cuttings and other species are more difficult and take a longer time to root than juvenile material (Lunguist & Torrey, 1984; Hartmann *et al*., 1990). In propagation of spineless *Acacia wrightii, for example,* rooting of semi-hardwood and hardwood cuttings was low (Bryan, Arnold, Lineberger & Watson, 2005).

Semi-hardwood cuttings

Leakey & Coutts (1989) defined apical cuttings as softwood or semi-hardwood cuttings with developing apex and young leaves, which may produce rooting promoters, but these could also inhibit rooting if present in excess.

According to Hartmann *et al*. (1990) many ornamental shrubs, such as *Pittosporum*, *Camellia, Rhododendron, Euonymous*, holly and evergreen azaleas are commonly propagated by semi-hardwood cuttings. A few fruit species such as citrus and olive can be propagated by semi-hardwood cuttings (Hartmann *et al*., 1990).

In some plant species such as spineless *Acacia wrightii*, apical cuttings rooted at high enough rates, and proved to be a commercially viable method of production (Bryan *et al*., 2005). Furthermore, Pijut (2004) found that the greatest rooting success on propagation of butternut tree by softwood cuttings than on hardwood cuttings. Mng'omba, du Toit, Akinnifesi & Venter (2007) found that *Pappea capensis* stem micro-cuttings in tissue culture are difficult to roots but they have good sprouting ability and leaf retention ability when pre-treated with ½ Murashige & Skoog (MS) growing media. In litchi, rooting was found to be the best (80%) in 25 mg/ml concentration of indole butyric acid (Das, Prakash & Bhalla-Sarin, 1999) after four weeks (Table 1.1).

Table 1.1 Effect of pulse treatment for 15 minutes with different concentrations of auxin on root formation from litchi shoots (cv. 'Bedana'), after 4 weeks of culture [Values are means of three replications with 20 shoots in each replication (Adapted from Das *et al*., 1999)]

Auxin (mg/ml)	Percentage of shoot responding	Mean no. of roots per shoot
NAA (1)	0	0b
NAA (5)	0	0b
NAA (25)	45	1.2 ± 0.8a
2-4D (1)	0	0b
2-4D (5)	0	0b
2-4D (25)	0	0b
IBA (1)	0	0b
IBA (5)	0	0b
IBA (25)	80	1.8 ± 0.8a

Means with the same letter do not differ significantly (P=0.05) from each other as indicated by one-way ANOVA followed by Tukey's multiple comparison test

1.1.2 Vegetative propagation by air layering

Air layering is a rooting method where the rooting medium is wrapped around the aerial stem, causing it to produce adventitious roots while the stem is attached to the mother plant (Macdonald, 1986; Thompson, 1989; Hartmann *et al*., 1990). This technique is one of the oldest methods of vegetative propagation which was used to propagate plants in China (Macdonald, 1986) and it is still being used to multiply plants, especially those which are difficult to root by cuttings and grafting (Thompson, 1989; Das, Basak & Das, 1996; Eganathan, Rao & Arnold, 1999). Philip (1984) indicated that air layering is easily accomplished without special facilities such as mist or polyethylene enclosures and it seems to be more applicable to small farms. The success of air layering as a practice in propagation is probably due, in part, to the effect of endogenous auxins accumulating at the base of the girdled shoots (Cameron & Thompson, 1969).

According to Mialoundama, Avana, Youmbi, Mampouya, Tchoundejeu, Mbenyo, Galomo, Bell, Kopguep, Tsebeng & Abega (2002), *Dacryodes edulis* (G. Don) H.J. Lam was identified as a difficult species to be multiplied vegetatively after several failures to propagate it through cuttings and grafting but the air layering method produced successful results. It has been noticed that root development may vary in different species. La Pierre (2001) reported success of 73.3% with the propagation of *Cecropia obtilsifolia.* Out of 60 stems prepared by air layering 44 stems produced adventitious roots after 14 days. In litchi, layers took 30-34 days to produce roots with one treatment; with another treatment roots were noticed after 50 days (Gowda, Shyamalamma & Prasad, 2006).

1.1.3 Seasonal effects

Seasonal timing in which cuttings are taken plays an important role in rooting and with many species there is an optimal period of the year for successful rooting

(Hartmann *et al*., 1990). The correct time to root cuttings is determined by cultivar, geographical location of the nursery, propagation facility and crop growing system (Macdonald, 1986). This was confirmed when high rooting percentages (Table 1.2) on bush tea were recorded in autumn and spring (Araya, 2005). Andres, Sanchez, Catalan, Tenorio & Ayerbe (2003) reported greater rooting percentage of *Colutea istria* (Bladder Senna) leafy stem cuttings collected in winter than those collected in autumn and winter cuttings produced the longest roots (Table 1.3).

Similar results were obtained with the rooting stem cuttings of four wing saltbush (*Atriplex canescens*), cuneate saltbush (*Atriplex cuneutu*), shadscale (*Atriplex conferfifolia*), spiny hopsage (*Grayiu spinosa*) and greasewood (*Sarcobatus vermiculatus)* (Richardson, Bakker, Crofts & Epps, 1979) where, regardless of rooting auxin application the season affected rooting of the cuttings. Juvenile *Ficus pumila* cuttings can be treated with IBA to overcome the seasonal fluctuation but with mature cuttings IBA makes no difference (Hartmann *et al*., 1990).

Table 1.2 Rooting percentages of bush tea cuttings 15, 20, 25 and 30 days after planting in summer, autumn, winter and spring (Adapted from Araya, 2005).

Days after planting	Rooting %			
	Summer	Autumn	Winter	Spring
15	25.0a	40.0a	5.0a	46.3a
20	45.3b	65.0b	21.7b	71.0b
25	-	75.0bc	28.8bc	67.6b
30	-	77.5c	40.0c	84.4c

Figures in a column followed by the same letter are not significantly different from each other (P>0.05), using Tukey's comparison test

Table 1.3 Effect of cutting origin, Indole butyric acid treatment and season of collection on rooting of *C. Istria* leafy stem cuttings in Madrid, Spain (Adapted from Andres *et al*., 2003)

	N	RP%	RN	RL (cm)	RDW (mg)	LDW (mg)
Cutting origin						
Apical	12	8.67a	3.06a	39.03b	19.4a	75.9b
Medial	12	13.67a	5.15ab	55.08b	83.1a	184.9b
Basal	12	13.58a	6.96a	110.50a	70.0a	343.7a
IBA						
0	18	5.06b	2.40b	61.10a	32.0a	161.3a
200	18	18.89a	5.91a	71.27a	72.5a	225.9a
Season						
Winter	18	15.17a	5.71a	120.23a	75.9a	249.1a
Autumn	18	8.78b	4.83a	13.60b	53.5a	176.7a
Overall Scores	36	11.97	5.29	69.45	65.2	214.2

N, number of plots of 16 cuttings per plot; RP, rooting %; RN, root number per rooted cutting; RI, longest root length per rooted cutting (cm); RDW, root dry weight per rooted cutting (mg); and LDW, leaf dry weight per rooted cutting (mg).

According to Gowda, Shyamalamma & Prasad (2006), litchi layers prepared in July (monsoons) produced the highest rooting percentage of 59.7 followed by June (monsoons) 51.8 and the lowest percentage was recorded during September (monsoons) 40.5. Eganathan, Rao & Anand (1999) found October (monsoons) to be the best time to initiate air layers of three mangrove species (*Excoecaria agallocha*, *Heritiera formes* and *Intsia bijuga*) followed by January.

1.1.4 Effects of exogenously applied auxins to promote rooting

In many species the formation of root initials can be enhanced by the application of externally applied plant growth regulators; to accelerate root initiation and development, to increase the rooting percentage, to increase the number and quality of roots produced per cutting and to increase the uniformity of rooting

(Halfacre & Barden, 1979). Plant growth regulators such indoleacetic acid (IAA), indolebutyric acid (IBA) and napthaleneacetic acid (NAA) are important in influencing root growth (Halfacre & Barden, 1979; Wiessman-Ben & Tchoundjeu, 2000) and can be applied alone or in combination (Halfacre & Barden, 1979).

According to Halfacre & Barden (1979), efficiency of rooting auxins substance depends on specific concentration range for an individual species. That is, high concentrations may injure or kill the base of a cutting or cause excessive callusing and low concentrations may be ineffective (Halfacre & Barden, 1979). Puri & Verma (1996) confirmed that high concentrations of auxins inhibited or slowed down the rooting ability of cuttings as well as triggering both growth and differentiation of roots. Pijut & Moore (2002) reported the greatest rooting of 22.2% and 27.8% on *Juglans cinerea* when hardwood cuttings were treated with 62 mM K-IBA and 74 mM IBA.

In vegetative propagation of mangrove tree species, Eganathan *et al*. (1999) reported maximum rooting when cuttings and air layers were treated with IBA alone up to 2500 ppm in all tree species. But in both *Heritiera fomes* and *Heritiera littoralis* maximum number of roots per cutting was obtained on cuttings treated with IBA and NAA (Das, Basak & Das, 1996). This was caused by the interaction of IBA and NAA promoting starch hydrolysis during root development and subsequently reduced the C/N ratio and increased the protein-nitrogen activity during the root primodial development (Das, Basak & Das, 1996). Auxin is frequently not the main, or only limiting factor in difficult-to-root species but that endogenous substances such as phenolic compounds (Davies & Hartmann, 1988) must be effective in synergism with auxin in inducing rooting (Gowda *et al*., 2006).

1.1.5 Phenolic compounds

Phenolic compounds are a group of secondary products universally present in higher plants. They participate in structure of plants and are involved in metabolic pathways, as well as in plant environment and plant pathogen interactions (Errea, 1998; Taiz & Zeiger, 2002). Phenolic compounds are formed in vesicles, which are derived from the rough endoplasmic reticulum, and they are stored in plant cell vacuoles (Errea, 1998) and most are derived from phenylalanine in the Shikimic acid pathway (Taiz & Zeiger, 2002). Some phenolic compounds are soluble in organic solvents. Carboxylic acids and glycosides are water soluble and others are insoluble polymers (Taiz & Zeiger, 2002).

When plants are cut and removed from mother plants, they are wounded and wounding influences oxidation process and release of phenolics from the plant cell vacuoles. Sometimes wounding is done to encourage rooting especially in layering when shoots are girdled to expose the inner parts and force them to produce roots. Gud, Gad & Haas (1988), Hartmann *et al*. (1990), and Errea (1998) reported that some phenols inhibit formation of roots while others are produced when trees are wounded in order to play a role in wound healing. The inhibiting phenolic compounds make propagation of plants to be difficult as initiation of roots does not occur and scion/rootstock combination becomes incompatible (Hartmann *et al.,* 1990; Errea, 1998; Usenik, Kriska, Vican & Stampar, 2006).

Bartolini, Fabbri & Tattini (1988) found that phenolic acids stimulated the formation of adventitious roots in grape rootstock cuttings. However, high concentrations of cinnamic acid in hardwood cuttings of *Chamaelaucium* inhibited root formation (Curir, Sulis, Mariani, van Sumere, Marchesini & Dolci, 1993). In a study by Errea, Garay & Marini (2000), different phenolics like phloroglucinol, catechin and p-coumaric acid were found in incompatible unions of apricot (*Prunus armeniaca*) when it was grafted on other *Prunus* species. Gur *et al*.

(1988) suggested a close correlation between root formation, phloroglucinol and phloridzin in certain apple rootstock clones *in vitro*. In contrast, phloroglucinol and phloridzin have been shown to promote the rooting of certain apple rootstock clones and to reduce rooting of others if applied with indole butyric acid (IBA).
Girdling and cutting of planting materials do not only promote production of phenolic compounds but improve carbohydrate accumulation in branches of trees (above the girdle).

1.1.6 Carbohydrates

Carbohydrates are very important basic building blocks of structural elements and energy supply for plant tissues (Taiz & Ezeiger, 2002; Correa, Palm, Schwambach & Fett-Neto, 2005) which are formed during the photosynthesis process. They are therefore important for root formation (Struve, 1981). Couvillon (1988) observed higher levels of total carbohydrates in an easy-to-root cultivar of chrysanthemum and it produced a greater number of roots than did the difficult to root cultivar. Hoad & Leakey (1996) stated that rooting success is associated with low pre-severance starch and water-soluble sugar concentrations and greater total water-soluble carbohydrates (TWSC). Correa *et al*. (2005) often considered exclusively the availability of carbohydrates as an energetic requirement to drive rooting and together with phytohormones (auxins), they are mentioned to be the major regulatory substances in the root development process. According to Hoad & Leakey (1996) rooting is associated with well maintained stem starch and an increase in stem total water-soluble carbohydrates during the propagation period.

Reserve of carbohydrates

Chalfun, Pasqual, Norberto, Dutra & Cavalcante-Alves (2003) mentioned that a richer reserve of carbohydrates correlates with higher rooting percentage as well as the cuttings' survival. Palanisamy, Ansari, Kumar & Gupta (1998) also reported a higher levels of total soluble sugars in the rooting zone in non-rooted

than in rooted cuttings of neem and karanj (Table 1.4). This is caused by depletion and utilization of sugars during differentiation and growth of adventitious roots.

Table 1.4 Total soluble sugar content (mg gfwt^{-1}) in the rooting zone of neem (*Azadirachta indica)*; 65 days after planting and karanj (*Pongamia pinnata*; 35 days after planting (Adapted from Palanisamy *et al*., 1998).

	Nature of the cuttings	Control	IBA	SD at P=0.05
Plant		Total soluble sugar content (mg gfwt^{-1})		
(a) Neem	RS	9.1	9.6	NS
	NRS	21.6	-	
	RWS	-	7.4	
SD at p=0.05		4.3	0.9	
(b) Karanj	RS	4.9	3.8	NS
	NRS	14.9	-	
	RWS	-	3.6	
SD at p=0.05		2.0	NS	

RS = rooted with sprouts, NRS = non-rooted with sprouts, RWS = rooted without sprouts, NS = non significant

According to Storey (1968), a high percentage of successful grafts can be obtained by using scions high in starch. High starch content can be promoted by girdling the stems or air layers (Table 1.5). It causes an interruption in downward translocation of organic materials such as carbohydrates, auxins and other growth factors from leaves and growing shoot tips (Erdogan & Smith, 2005). The wounding stimulates cell division and production of root primordia on cuttings

(Erdogan & Smith, 2005) caused by accumulation of carbohydrates and auxin as well as formation of a new sink area (Hartmann *et al*., 1990).

Table 1.5 Effect of girdling on carbohydrate accumulation in litchi (*Litchi chinensis*) cuttings (Adapted from Storey, 1968).

Percent dry weight	Girdled branch	Non-girdled branch
Starch	11.40	0.40
Total Sugar	2.10	1.68
Protein nitrogen	1.14	1.16
Soluble nitrogen	0.10	0.12

Gunes (1999) reported a decrease in concentration of both starch and soluble sugars for all types of walnut cuttings. This was recorded after callusing or bud burst, but found no correlation between callusing and carbohydrate level. In loblolly pine, Rowe, Blazich & Weir (1999) were able to relate carbohydrates and initial mineral nutrient, particularly nitrogen fertilizer. In rooting of chrysanthemum cultivars, an easy to root cultivar had higher level of carbohydrates and produced a greater number of roots than did the difficult to root cultivar (Convillon, 1988). Carbohydrate levels in cuttings at collection time are reported to be important in rooting because cuttings in a rooting bed do not produce new carbohydrates; instead they use the stored ones.

1.1.7 Flowering

Pappea capensis trees (Fig. 1.1) growing at the Experimental Farm of the University of Pretoria produce flowers in summer. The staminate flowers are initiated first and then followed by pistillate flowers. Palmer & Pitman (1961) observed that male flowers are borne in panicles while the female flowers in a bunch. *Pappea capensis* was related to be a monoecious plant that produces male flowers and then female flowers either on the same inflorescence or on

different inflorescences (Fivaz & Robbertse, 1993). According to Crane, Balerdi, Campbell & Knight (1998) there are three types of flowers in litchi: two male (M1 and M2), one female (F) and M1 flowers open first, then followed by female flowers and M2 flowers are the last flowers to open. Similarly, longan produces three types of flowers, staminate, pistillate and hermaphrodite, with pistillate flowers setting fruit (Lin, Zheng, Luo, Song & Guan, 2001).

Influence of temperature on flowering

Floral induction in litchi (*Litchi chinensis*) is influenced by relatively low temperatures but water deficits have no impact on flowering (Batten & McConchie, 1995; Menzel, 2000). Moreover, lower temperatures lead to a higher percentage of Type II flowers (Nakasone & Paull, 1998). These flowers are morphologically hermaphrodite, functioning mostly as female with well-developed two carpel pistils and a two-lobed stigma. Observations indicated that the ratio of functional females (Type II) to functionally staminate flowers (Types I and III) varies with cultivar (Table1.6) in litchi (Nakasone & Paull, 1998). But in the case of *Pappea capensis,* lack of water may cause the tree to produce only male flowers or fail to produce flowers at all (Fivaz & Robbertse, 1993). Lin *et al*. (2001) reported an increase in concentrations of endogenous hormones and polyamines during floral sex differentiation in longan which might influence production of certain types of flowers.

Table 1.6 Distribution of the three sex types of flowers per panicle of two litchi cultivars (Nakasone & Paull, 1998)

		Functional flower type (%)			
Cultivar	Flowers per Panicle	I	II	II	Potential fruiting flowers (%)
Groff	964	14	20	66	20
Heiye	1042	22	19	59	19

Menzel & Simpson (1992) mentioned that higher numbers of female flowers in litchi were associated with maximum temperatures of 18℃ during flower development but high temperatures (23℃) reduced the number of female flowers. In these types of trees, production of many female flowers results in high yields.

Even though temperature plays the major role over sex expression, monoecious and dioecious conditions are reported as species characteristics which are under basic hereditary control (Greulach, 1973). Despite temperature and hereditary control, there are other factors which can greatly influence the change from one phase to another such as light, hormones and nutrition.

Figure 1.1 *Pappea capensis* (Tree No. 1) growing at the Experimental Farm of University of Pretoria

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CHAPTER 2

ROOTING OF *PAPPEA CAPENSIS* STEM CUTTINGS

2.1 Abstract

Pappea capensis was selected by scientists as a potential tree for commercial biodiesel production. It is known that the tree can be propagated by means of seed (Venter & Venter 1996). However, cloning trees using vegetative propagation would be preferable. Therefore, the use of stem cuttings was the first option studied because clonal propagation of most trees and shrubs is effective by stem cuttings, although stem cuttings of some species it remains difficult to initiate adventitious roots. This study was therefore carried out to determine the rooting ability of *Pappea capensis* stem cuttings so that cuttings can be used for rapid multiplication in commercial production. Cuttings were collected in spring and autumn and struck in a mist chamber with bottom heat maintained at ±23ºC with or without pre-treatments with rooting auxin (indole butyric acid). In experiment 2, auxin treatment was not applied to five of the cuttings and then applied at intervals to some of the cuttings from 0-5 weeks after planting. The influence of girdling of shoots as a pre-treatment was also investigated. Rooting performance of stem cuttings was found to be generally poor. Thus, in this investigation, propagation of *Pappea capensis* by means of stem cuttings was found to be unsuccessful.

Key Words: *Pappea capensis,* vegetative propagation, stem cuttings, Seradix ® No. 2 powder (IBA)

2.2 Introduction

Stem cutting propagation is commonly used in commercial production for rapid multiplication of plant materials. Its advantage as compared to other forms of vegetative propagation lies in its ability to create a large number of young plants from a single parent, and is therefore a useful technique for conservation of endangered plants as well as maintaining uniformity of genotypes. Difficulties with propagation of certain woody plants from stem cuttings may limit their domestication and commercial cultivation (Dawson & King, 1994). The reason behind this is that rooting success of stem cuttings is dependent on many factors, such as type of wood, hormonal (endogenous and exogenous) influence and the season when cuttings are collected from the mother plant. Other contributing factors in adventitious root formation are physical and environmental factors (Hartmann *et al*., 1990). Therefore, several experiments were conducted to evaluate the rooting potential of stem cuttings of *Pappea capensis* by applying indole butyric acid (IBA) on both semi-hardwood and hardwood cuttings taken in spring and autumn. In some experiments the tree shoots were girdled to improve carbohydrate status of stem cuttings and rooting auxin application was delayed.

2.3 Materials and methods

2.3.1 Experimental site and plant material collection

The stem cuttings were collected in spring season (2006 and 2007) and in autumn season (2007 and 2008) from two mature trees (over 30 years old) at the Experimental Farm of the University of Pretoria, South Africa (25º 45' S, 28º 16' E and 1372 m above sea level). One tree (Tree No 1) bears fruits every year although in 2006 the flowers were male and then followed by female flowers. The other tree (Tree No. 2) produces predominantly male flowers, and it did not bear fruits during the study period although the remains of fruits were found

underneath the tree, showing that it did bear fruits the previous year. The cuttings were collected in the morning in buckets filled with water to reduce water loss and to keep them cool and turgid until striking in the mist bed.

The mist bed that was used was 5 m long, 1.5 m wide and 1 m high supplied with an automatic misting system operating through misting nozzles set to switch on at 10 seconds every 5 minute. Throughout the experimental period, the temperature and relative humidity in the greenhouse were recorded using a digital thermohygrograph (HOBO®Temp/RH (H14-001) data logger and light intensity was recorded weekly from striking to harvesting time, using a linear PAR/LAI ceptometer (AccuPAR (Model PAR-80) logger net 3.2 Campbell Scientific Inc., CR200). The measured mean minimum and maximum temperatures, relative humidity and light intensity during spring and autumn were 22°C, 57%, 297µmol m^2s^{-1} and 23°C, 79%, 364 µmol m^2s^{-1}, respectively.

2.3.2 Experimental design and treatments

Three experiments were conducted on stem cuttings. Experiment 1 consisted of 2 x 2 x 2 x 2 treatments [2 trees, 2 types of cuttings, 2 seasons and 2 auxin treatments, Seradix® No. 2 talc (active ingredient is 0.3% 4-indole-3-butyric acid); Bayer, Pretoria, South Africa], and 21 cuttings for each treatment. The 2^4 factorial experiments were laid out as a randomized complete block design with six replications (Fig. 2.1). The second experiment consisted of 2 x 2 x 5 treatments where cuttings were collected randomly in spring and autumn seasons from the same *Pappea capensis* trees used in the first experiment and in this case the IBA treatment was delayed on five cuttings of each treatment (no IBA, 5 week intervals).

The propagation medium was a 1:1 (v:v) mixture of pasteurised fine and coarse silica sand. The medium was filled into seedling trays with 5 x 3 x 4.5 cm (length, width and depth) cells (a total of 98 cells/polystyrene tray) the day before

planting. The trays were put under the mist system set at 5 minute intervals for 10 seconds and left over night to wet the rooting medium thoroughly.

Experiment 1 Rooting of apical and basal stem cuttings

The semi-hardwood (apical) and hardwood (basal) cuttings (10 – 12 cm long) were taken randomly from Trees No. 1 and No. 2 at the Experimental Farm of the University of Pretoria early in the morning when the plants were still turgid and plastic bags with water were used to keep the cuttings turgid until they were planted (Agbo & Omaliko, 2006). Cutting collections were done in spring and autumn seasons. Basal (proximal) leaves were removed and only four (distal) leaves were left to reduce the evapotranspiration rate during rooting. One third of each of these remaining leaves was removed,using sharp secateurs. The entire stem cuttings were then dipped in Benomyl (50mg/ℓ) (Morgenson, 1992; Prat, Botti & Palzkill, 1998) to control potential fungal problems during rooting. The basal (2 cm) part of the cutting was dipped in dry rooting powder (Seradix® No. 2, 0.3% 4-indole-3-butyric acid). Observations were made throughout the entire experiment and harvesting was done eight weeks (60 days) after striking.

Experiment 2 Effect of delaying basal auxin treatment on cuttings collected from girdled *Pappea capensis* branches

This experiment was also conducted during the spring and autumn seasons from 2007 to 2008. Tree branches of Trees No. 1 and No. 2 were girdled six weeks before cuttings were made to improve carbohydrate status. Only apical stem cuttings were collected from the girdled branches and then cut into different lengths (5 cm and 10 cm). The bottom leaves were removed, leaving two pairs of leaves at the top. The leaves were further trimmed into half size to reduce leaf transpiration. After planting, the cuttings were sprayed with a fungicide (Benomyl® (5g per 10ℓ); Bayers, Pretoria, South Africa).

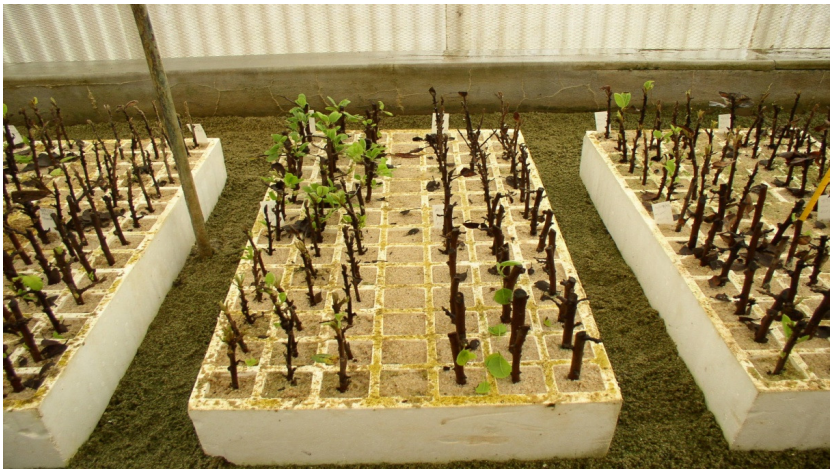
In this experiment, IBA rooting powder (Seradix® No. 2) was not applied to stem cuttings (control). In some stem cuttings rooting auxin treatment was applied immediately at striking (0 week) and for the rest of the stem cuttings auxin treatment was delayed at weekly intervals for six weeks (week 1, week 2, week 3, week 4 and week 5). The auxin was applied by removing the stem cutting from the rooting medium, rinsing the base of the cuttings with water followed by quick dipping in Seradix® No. 2. After the rooting powder treatment the cuttings were replaced back into the same cavity in the tray. Stem cuttings were studied for after three weeks after the last IBA application. The purpose of delayed hormonal treatments was to find out the convenient time for rooting auxin application for induction of adventitious roots.

Experiment 3 Rooting of *Pappea capensis* coppice stem cuttings

Ten apical stem cuttings were collected from a *Pappea capensis* tree that was cut down severely to the ground level and allowed to produce new shoots. The cuttings were treated and placed in the rooting medium as described in Experiment 1. Five cuttings were treated with Seradix® No. 2 powder and the other five were not treated with rooting Seradix® No. 2.

2.3.2 Data Analysis

Data were analysed using the PROC GLM (General Linear Model) procedure in the S.A.S (Statistical Analysis System version 9.2). ANOVA (Analysis of variance) was done to determine significant differences of the means using the Least Significance Difference (LSD) test.


A

B

Fig. 2.1 Illustration of experimental layout used during the propagation of *Pappea capensis* stem cuttings on the mist bed in spring season (A) in 2006 and autumn cutting experiment on the mist bed (B) in 2007.

2.4 Results

Attempts to induce adventitious roots from stem cuttings were unsuccessful. Most of the cuttings were still alive at harvesting and had produced callus; the others were dead at harvest. The majority of the cuttings lost their leaves soon after planting. This was then followed by sprouting of buds on cuttings. Most of the cuttings from the spring season sprouted, while only a few of the cuttings struck in autumn sprouted and only five stem cuttings produced roots.

Experiment 1 Rooting of apical and basal stem cuttings

No roots were observed on cuttings taken in spring or in autumn. During harvesting and data collection (in 2007), some of the stem cuttings were still alive while others were dead. Most of the leaves were lost relatively early during the rooting process. This was followed by breaking of buds and sprouting of new leaves. Some stem cuttings wilted and died within 60 days of being placed in the mistbed (Table 2.1). All the stem cuttings collected from Tree No. 2 were found to be alive, whereas certain cuttings from Tree No. 1 were dead.

Higher survival percentages of 97% and 90% were also observed on hardwood (medial) cuttings from IBA treated and untreated cuttings collected from Tree No. 1, respectively. However, lower survival percentages were found on semi-hardwood (apical) cuttings of Tree No. 1 with (52%) and without IBA treatment (63%)

Table 2.1 Survival and sprouting percentage, after 80 days of *P. capensis* stem cuttings which were made in spring 2006 and placed in the mistbed for rooting

Tree	Cutting type	Survival %		Sprouting %	
		No IBA	IBA	No IBA	IBA
No. 1	Apical	63c	52c	43b	21e
	Basal	97a	90b	37b	67f
No. 2	Apical	100a	100a	40c	50a
	Basal	100a	100a	48a	30d
	Average	90	86	42	42

Means within a column and treatment with similar alphabetical letters are not significantly different from each other at $P<0.05$.

2.4.2 Callus formation on *Pappea capensis* stem cuttings

No successful rooting was observed in either spring 2006 or autumn 2007. However in 2007 some of the cuttings produced callus at the basal cut end. During the spring season callusing was very poor and very small amounts were formed at the basal ends of the cuttings (Table 2.2). There was no significant difference between callusing of stem cuttings from Tree No. 2 (14%) and Tree No. 1 (9%). Production of callus was significantly higher (21%) than in the control treatment compared to where Seradix® No. 2 (2%) was applied. The basal cuttings tended to form more callus than the apical cuttings.

Cuttings taken during autumn showed significant differences ($P<0.05$) due to tree treatment as well as IBA treatment. Sixty percent and 30 percent of stem cuttings obtained from Tree No. 2 and Tree No. 1 produced callus, respectively (Table 2.2). However, many (80%) of the stem cuttings not treated with rooting powder produced callus.

After leaf shedding, sixty percent of stem cuttings sprouted from buds which were dormant during collection. It was observed also that the leaves produced from the sprouted buds were weak and dropped easily.

Table 2.2 Callus formation on stem cuttings taken in spring 2006 and autumn 2007 from *Pappea capensis* trees (Tree No. 1 and No. 2)

		Callused stem cutting %	
Treatments		Spring	Autumn
Tree	Tree No. 1	9a	30b
	Tree No. 2	14a	60a
	LSD $_{(0.05)}$	8.34	13.19
	CV (%)	25	10
Hormone	Control	21a	80a
	Seradix® No. 2	2b	10b
	LSD $_{(0.05)}$	8.34	13.19
	CV (%)	12	12
Wood	Hardwood (basal)	15a	-
Type	Semi-hardwood (apical)	8a	-
	LSD $_{(0.05)}$	8.34	-
	CV (%)	20	

Means within a column and treatment with similar superscript letters are not significantly different from each other at $P<0.05$.

2.4.3 Experiment 2 Effect of delaying hormone on cuttings collected from two girdled *Pappea capensis* trees

Delaying IBA application (Seradix® No. 2) on cuttings did not improve rooting percentage (Table 2.3). The results showed better stem callus, survival and rooting percentage when the auxin was applied after the first week of striking on 10 cm long stem cuttings collected from Tree No. 1. Similar results were also observed on delaying IBA application by two and four weeks but no roots were produced.

However a very high callus percentage (42%) of stems obtained from Tree No. 2 (5 cm long) was found on the control and when auxin was delayed for one week after striking. Most of the stem cuttings that survived had been treated with IBA after three and four weeks.

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Table 2.3 Effect of delaying IBA application to stem cuttings of two *P. capensis* trees on callusing, rooting and survival of cuttings

Trt	Stem cutting length (cm)	Tree No. 1						Tree No. 2					
		Callused %		Survival %		Rooting %		Callused %		Survival %		Rooting %	
		Aut	Spr	Aut	Spr	Aut	Spr	Aut	Spr	Aut	Spr	Aut	Spr
C	5	0c	0c	0c	20a	0b	0b	42a	0c	0c	20a	0b	0b
	10	0c	0c	0c	20a	0b	0b	0c	0c	0c	21a	0b	0b
W0	5	0c	0c	0c	18a	0b	0b	0c	0c	0c	20a	0b	0b
	10	0c	0c	0c	20a	0b	0b	0c	0c	0c	20a	0b	0b
W1	5	0c	0c	0c	17a	0b	0b	42a	0c	0c	20a	0b	0b
	10	25b	0c	25b	21a	4.2a	0b	0c	0c	0c	20a	0b	0b
W2	5	0c	0c	0c	20a	0b	0b	0c	0c	0c	20a	0b	0b
	10	29b	0c	29b	18a	0b	0b	0c	0c	0c	20a	0b	0b
W3	5	0c	0c	0c	19a	0b	0b	0c	0c	0c	19	0b	0
	10	25b	0c	25b	17a	0b	0b	0c	0c	42a	20a	4.2a	0b
W4	5	0c	0c	0c	18a	0b	0b	0c	0c	0c	20a	0b	0b
	10	29b	0c	29b	21a	0b	0b	0c	0c	42a	20a	4.2a	0b
W5	5	0c	0c	0c	20a	0b	0b	0c	0c	0c	20a	0b	0b
	10	0c	0c	0c	0c	0b	0b	0b	0c	0 c	20a	0	0b
	Average	7.71	0	7.71	19	0	0	0	0	0	7.29	0.6	0
	CV (%)	13.2	15.5	10.7	8.9	17	20	13.2	15.5	10.7	8.9	17	20

Means within a column and treatment with similar alphabetical letters are not significantly different from each other at $P<0.05$. Trt = treatment, W = weeks, Spr = spring, Aut = autumn, C = control.

2.4.3 Rooting of coppice stem cutting

The stem cuttings failed to produce roots callus and they all died.

2.5 Discussion

In experiment 1, the investigation indicated that many attempts to root *Pappea capensis* (Jacket plum) stem cuttings failed, irrespective of the type of cuttings used (semi-hardwood or hardwood), rooting auxin treatments, girdling and whether the cuttings were taken in spring or autumn. However, very few of the cuttings were able to produce callus and roots from Experiment 2. Callus production was found to be better and more visible in cuttings collected in autumn than in those collected in spring which means the season also played an important role in callus formation.

Similar observations were made by Mng'omba *et al*. (2007) in the tissue culture where difficulties in rooting of micropropagated micro stem cuttings of *Pappea capensis* were reported. Furthermore, Das *et al*. (1999) also reported unsuccessful rooting of litchi cuttings (cv Bedana) when IBA and NAA were applied on stem cuttings collected in spring and autumn.

In most cases rooting ability of most species is affected by type of wood cutting, type of medium used, time of the year and presence or absence of hormone when cuttings are collected from the stock plant (Hartmann *et al*., 1996). Davies & Hartmann (1988) reported that poor rooting on woody species was closely correlated with extensive sclerification caused by thick lignified walls of sclerenchyma tissues, between the phloem and cortex outside the point of origin of adventitious roots. The situation hinders the growth of roots and such cuttings are then determined to be difficult to root. Easy-to-root species form fewer sclerenchyma cell layers between the phloem and where the roots originate (Hartmann, Kester, Davies & Geneve, 1997). Better rooting was recorded on

stem cuttings taken from young trees rather than old trees of Eastern red cedar (Henry, Frank & Hensley, 1992). In the present experiment, stem cuttings were obtained from trees (two trees) over 30 years old but also from a coppicing stump, but in all cases rooting was unsuccessful. According to Hartmann *et al*. (1990) some species do not propagate easily with mature and highly lignified cuttings and there is considerable variability among different species regarding the ease of rooting of cuttings. Even though juvenile cuttings are known to produce roots easily, *P. capensis* coppices did not produce roots.

The beneficial effect of treating cuttings with rooting auxin was recorded for stem cuttings of *Carolina hemlocks*, Atlantic white cedar, *Arbutus andranhne*, 'Yoshino Japanese cedar (*Cryptomeria japonica*) and *Leucospermum* (Hensley, Blazich & Snelling, 1994; Jull, Warren & Blazich, 1994; Al-Salem & Karam, 2001; Jetton, Frampton & Hain, 2005; Rodriguez-Perez, Leon-Hernandez, Vera-Batista, Rodriguez- Hernandez & Rodriguez-Herrera, 2006). For *Pappea capensis*, application of rooting auxin did not significantly show a positive response on root initiation. A similar case was also reported for *Genetum africana*, where insignificant differences were found in rooting percentage when rooting auxins were used (Shiembo, Newton & Leakey, 1996). When rooting auxins were applied to some difficult-to-root plants, rooting differences were reported but some plants do not respond well to rooting auxin applications (Hartmann *et al*., 1990).

Failure of a high percentage of the cuttings to root in this study could be due to early loss of leaves and the negative interaction of endogenous and exogenous auxins. Likewise, the activity of parenchyma cells at the basal portion of stem cuttings could have been inhibited by rooting hormone (Seradix® No. 2) if the concentration is at toxic levels. This occurrence could explain the low survival and sprouting percentage of auxin treated cuttings in autumn. Hartmann *et al.* (1990) confirmed that an effective, non-toxic concentration of rooting auxin has been used if the basal portion of the stem shows some swelling, callusing and profuse root production just above the base of the cutting. Moreover, auxin

treatments and rooting under mist cause cell expansion and cell proliferation in the cortex, phloem and cambium, resulting in breaks in continuous sclerenchyma rings (Karisantini, Johnston, William & Beveridge, 2006).

Swamy, Puri & Singh (2002) noticed a difference in rooting of *Robinia pseudoacacia* when NAA (500 mg/l) produced higher rooting percentages in juvenile (83.3%) and mature (66.6%) cuttings and *Grewia optivia* treated with IBA (250mg/l) produced high rooting percentages in juvenile (80%) and in mature (70%) cuttings. This is in line with the fact that a particular type of rooting auxin is effective in enhancing rooting production in particular species.

Another factor can be seasonal timing or the season of the year in which cuttings are taken, which can play an important role in successful rooting. This is applicable to certain plant species where there is an optimal period of the year for rooting but certain plant cuttings can be taken and rooted at anytime of the year (Hartmann *et al*., 1990). Seasonal effect was clearly visible in this study as some *Pappea capensis* stem cuttings produced more callus in autumn than in spring. In spring, fewer stem cuttings died in each treatment as compared to autumn and it was also observed that the *Pappea capensis* trees lost most of their leaves and sprouted (60%) in spring while few leaves were lost in autumn but cuttings did not sprout as much. Production of root initials or callusing of *Pappea capensis* in autumn could be due to carbohydrates and endogenous growth promoters that were available in stem cuttings during flowering in spring and potential fruit formation in autumn (February to May). However, Araya (2005) mentioned that cuttings taken at flowering stage had the opposite effect on vegetative propagation on bush tea (*Athrixia phylicoides*). This rooting variation occurrence due to different seasons was also obvious in both *Robinia pseudoacacia* and *Grewia optiva* (Swamy, Puri & Singh, 2002).

2.6 Conclusion

The basal and apical stem cuttings from *Pappea capensis* collected in spring and autumn did not successfully form adventitious roots. With and/or without application of exogenous auxin (Seradix® No. 2) treatments there were no significant differences found. However, during autumn season callus and very few stem cuttings produced roots. It is clear that seasonal effect plays a major role in rooting of *Pappea capensis* stem cuttings because stems produced more callus in autumn season. Rooting of *Pappea capensis* was very poor when the cuttings were obtained from mature trees because regeneration is very low in old trees. Furthermore, rooting stem cuttings in spring and autumn season cannot be chosen as the appropriate seasons for vegetative propagation of *Pappea capensis* trees. Secondly, delaying application of rooting auxin (IBA) on *Pappea capensis* stem cuttings did not improve rooting ability as reported by in Eucalyptus stem cuttings study.

In the preliminary studies reported here vegetative propagation by stem cuttings was found to be unsuccessful and alternative vegetative propagation techniques need to be tried for *Pappea capensis*.

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CHAPTER 3

ROOTING OF *PAPPEA CAPENSIS* BY AIR LAYERS

3.1 Abstract

Air layering was used as an alternative vegetative propagation technique as *Pappea capensis* stem cuttings were difficult to root in a previous study. In this experiment the effects of IBA treatment (Seradix® No. 2), tree gender, canopy quadrants (south, east, west and north) and season (spring and autumn) on rooting of air layers were studied. Branches with a diameter of 2 cm were selected and ring barked on two mature *Pappea capensis* trees (Tree No. 1 and No. 2). Certain air layers were dusted on the distal end of the girdle with Seradix® No. 2, and others were not. The girdled air layers were wrapped with moist peat moss, plastic and foil. Indole butyric acid treatment on air layers showed inconsistency but girdled shoots of Tree No. 1 showed the best rooting ability in spring. Higher rooting percentages (100%) were obtained on the northern side of Tree No. 1 in both spring and autumn seasons.

Keywords: *Pappea capensis*, air-layers, Seradix® No. 2, season, quadrants

3.2 Introduction

Air layering (marcottage) is an alternative vegetative propagation technique, which is used to multiply some plants for cultivation. Hartmann *et al*. (1990) mentioned that the technique is used to propagate many tropical and subtropical trees and shrubs, including litchi and Persian lime (*Citrus aurantifolia*). Air layering involves the girdling of the stem to expose the cambium and the application of rooting auxin to the exposed wound to stimulate root initiation. In

some plants air layers root easily without rooting auxin application but other plants are difficult to produce roots when a rooting auxin is not applied. In most plant species root formation is influenced by time and phytochemicals such as phenols and carbohydrates in the planting materials (Hartmann *et al*., 1990).

There are no known reports in the literature for clonal propagation of *P. capensis* by air layering. The propagation of *Pappea capensis* by stem cuttings, as discussed in Chapter 2, was unsuccessful. Therefore, the overall objective of this study was to determine if vegetative propagation by air layering could be developed as a practical alternative to stem cuttings and seed propagation. The effect of different parameters on rooting was investigated:

- Tree gender
- Season (spring and autumn)
- Seradix ®No. 2
- Canopy orientation (east, south, west, and north)

The effect of pytochemicals (carbohydrates and phenolics) on air layering was also studied, but a separate chapter (Chapter 4) is devoted to this topic.

3.3 Materials and methods

3.3.1 Experimental site

The experiment was conducted at the University of Pretoria's Experimental Farm. Two *P. capensis* trees planted at the Experimental Farm (25º 45' S, 28º 16' E and 1372m above sea level) were used. In spring, the experiment coincided with fruiting and in autumn, with flowering. During this study one tree produced male flowers (Tree No. 2) and the other tree female flowers (Tree No. 1). Throughout the duration of the experiment, the temperature, light and relative humidity were recorded using Campbell® Scientific Inc. logger net (3.2) CR 200 placed about 10 m from the trees. The measured mean average temperature, relative humidity,

rainfall and light intensity during spring and autumn were 23°C, 66%, 20 mm, 332 µmol m^2s^{-1} and 22°C, 39%, 500 mm, 277 µmol m^2s^{-1}, respectively.

3.3.2 Experimental design and treatments

The experiment was a split-plot design with two treatments (Tree No. 1 and Tree No. 2) and air layering with or without Seradix® No. 2 (containing 0.3% indole-3-butyric acid (IBA). Air layering was executed on branches (about 20 mm thick) and 60 cm from the tip of the shoots in four quadrants (north, south, east and west) of each tree. There were 5 air layers (replications) in each quadrant for each of the two treatments (IBA and control) on each tree.

The debarked rings on the 80 randomly selected branches were 2 cm in width according to recommendations of Gowda, Shyamalamma & Prasad (2006). In the rooting treatment the exposed cambium layers were dusted with Seradix® No. 2 using a soft small paint brush, while others were left untreated. According to recommendations of Zee, Nagao, Nishina & Kawabata (1999), the ring barked areas were covered with a moist rooting medium (peat moss) to keep the ring barked area moist and to allow root initiation. The rooting medium was then covered with clear plastic bags (38 cm x 25 cm) to hold the medium firm in position as well as keeping the medium moist for a longer period during the experiment. The open ends of the plastic bags were secured with plastic clamps on each end. Aluminum foil was wrapped over the plastic in order to prevent light to reach the developing roots (Fig 3.1). In autumn, the rooting medium dried quickly and it was then kept wet by regularly injecting 20 ml water once a week into the rooting media. Root growth was monitored at regular intervals.

3.3.3 Data Analysis

Data were analysed using the PROC GLM (General Linear Model) procedure in the SAS (Statistical Analysis System version 9.1). ANOVA (Analysis of Variance) was employed to determine significance of treatment effects and to calculate least significant differences (LSD) at P≤0.05.

Fig. 3.1 Illustration of air layers on *Pappea capensis* Tree No. 2 during the study at the Experimental Farm of University of Pretoria

3.4 Results

3.4.1 Rooting success of *P. capensis* air layers

Root initiation in spring was first noticed through the plastic after 4 weeks, but in the majority of the air layers, roots were noticed after 7 weeks for both trees. In autumn, the roots appeared later than 7 weeks. During the spring season, rooting percentage of air layers prepared on Tree No. 1 was significantly ($P < 0.05$) higher than those prepared on Tree No. 2. No significant difference in rooting percentage was observed in autumn (south) between the two trees (Table 3.1) where auxin was applied.

The average rooting percentage of air layers in spring was not improved by application of rooting IBA but the different situation was found in autumn, where most of the air layers treated with Seradix® No. 2 produced roots. In the control treatment, the rooting percentage of air layers from both trees in spring was significantly higher than air layers from the two trees in autumn. However, air layers treated with Seradix ® No. 2 in spring were only significantly greater in rooting percentage for Tree No. 1, but no differences were observed between autumn and spring air layers of Tree No. 2 (Table 3.1).

Untreated north, west and east quadrant air layers of Tree No. 1 rooted more in spring season than air layers from autumn season. But in autumn, Tree No. 1 IBA treated air layers resulted in better rooting on the south and north quadrants.

Table 3.1 Rooting percentage of *Pappea capensis* air layers in spring and autumn

Orientation		Tree No. 1		Tree No. 2	
		Spring	Autumn	Spring	Autumn
East	Control	100a	40b	80ab	20b
	Seradix® No. 2	60b	*	20d	0c
West	Control	100a	*	60b	0c
	Seradix® No. 2	60b	0d	40c	20b
South	Control	40c	20c	40c	0c
	Seradix® No. 2	80ab	40b	0e	40a
North	Control	100a	60a	60b	0c
	Seradix® No. 2	100a	0d	0e	40a
Average	Control	85	30	60	5
	Seradix® No. 2	75	10	15	25
CV (%)		9.9	17.5	9.9	17.5

Means with the same letters within a column are not significantly different from each other at $P < 0.05$

Seasonal variations in rooting of *Pappea capensis* air layers in both spring and autumn seasons were distinct, as shown in Fig. 3.2. There were, however, significant differences found in air layers treated with IBA and without IBA during spring (Fig. 3.2A and 3.2B). Many roots were observed on control than on Seradix treated air layers in spring. Irrespective of hormone application, very poor rooting was observed in autumn season (Fig. 3.2C and 3.2D). It is interesting to note that sprouting of leaves was visibly better in spring than in autumn.

Fig. 3.2 Air layers of *P. capensis* without IBA application and with IBA collected from the northern quadrant of Tree No. 1 in spring (A and B) and autumn (C and D) seasons. (F = Tree No. 1, N = North, W = -IBA and H = +IBA)

3.3.2 Root length, number and dry mass

Root length in *P. capensis* was not affected by IBA treatment in spring because the longest roots were observed on untreated air layers on the west, south and north sides of the tree canopy (Table 3.2). IBA treatment significantly influenced root length in spring on both trees, but in autumn season insignificant results were obtained (Table 3.2). It is important to note that air layers made in spring

produced long roots than air layers made in autumn season. Furthermore, the longer roots were observed on air layers that were made on Tree No. 1 compared to those on Tree No. 2.

As in the case of root number, in general air layers made in spring produced more roots than those made in autumn and air layers on Tree No. 1 outperformed those on Tree No. 2 by far. Higher numbers of roots per layer were observed on the southern quadrant (18.8) and northern quadrant (18.4) of Tree No. 1 in spring. However, very few roots were produced in autumn compared to those in spring season on both trees. There were no significant differences on root number due to IBA application. According to Table 3.2, air layers without IBA produced many roots than where IBA was applied. The highest average root number was obtained in spring than in autumn on both trees.

The highest figures for root length, root number and root dry mass were obtained in the spring air layers on Tree No. 1 without hormone treatment. Practically no roots were formed in the air layers made in autumn and thus the figures on root length and dry mass are not discussed here. The hormone treatment had no beneficial effect on either root number or length in the spring air layers, although it had a slight positive effect in the autumn air layers. The position of the air layer on the canopy also played a role. Rooting of air layers on the northern side of the tree canopy was greater than on the southern and eastern sides with an intermediate rooting performance on the western side of the canopy.

Table 3.2 Mean root length, root number and root dry mass of *P. capensis* air layers made in both spring and autumn seasons

		Root length (cm)				Root number				Root dry mass (g)			
Orientation	IBA Trt	Tree No. 1		Tree No. 2		Tree No. 1		Tree No. 2		Tree No. 1		Tree No. 2	
		Spr	Aut	Spr	Aut	Spr	Aut	Spr	Aut	Spr	Aut	Spr	Aut
East	Control	47.0abc	0a	30.6abc	4.2a	6.8abc	0a	6.2abc	1.0a	0.09a	0a	0.10a	0a
	Seradix® No. 2	61.4abc	0.8a	16bc	0a	4.6abc	0.8a	1.2bc	0a	0.07a	0a	0.02a	0.006a
West	Control	74.6abc	0a	25bc	0a	15abc	0a	3.2abc	0a	0.13a	0a	0.04a	0.008a
	Seradix® No. 2	36.4abc	1.0a	24bc	0.3a	7.4abc	0a	4.0abc	0.4a	0.05a	0.002a	0.05a	0a
South	Control	38.0abc	10a	9.4bc	0a	8.2abc	0.4a	0.4c	0a	0.04a	0a	0a	0a
	Seradix® No. 2	36.4abc	0.8a	0c	0.4a	18.8a	1.2a	0c	0a	0.16a	0.02a	0a	0a

North	Control	105.4a	2.4a	1.2bc	0a	18.4bc	1.0a	0c	0a	0.20a	0a	0a	0a
	Seradix ® No. 2	81.0ab	0a	0c	1.6a	14.6abc	0a	0c	0.8a	0.16a	0a	0a	0a
Average	Control	66.25	3.1	16.55	1.05	12.1	0.35	2.45	0.25	0.115	0	0.035	0.002
	Seradix ® No. 2	53.18	0.65	10	0.575	11.35	0.5	1.3	0.3	0.11	0.006	0.018	0.002
	CV (%)	10.8	10.7	10.8	10.7	8.9	11.6	8.9	11.6	17.5	20.9	17.5	20.9

Means within a column and treatment with similar alphabetical letters are not significantly different from each other at $P < 0.05$. Trt = treatment, W = weeks, Spr = spring, Aut = autumn.

3.5 Discussion

In this study, *Pappea capensis* air layers produced roots after four to seven weeks in the spring season. In comparison with the autumn season, the spring season was found to be the best period of propagating *Pappea capensis* (Jacket plum) when using the air-layering technique because very high rooting percentages within a shorter period were obtained in spring compared to the performance in autumn. In autumn, very few roots formed, and they appeared later than seven weeks after girdling. Mwang'ingo, Teklehaimanot, Lulandala & Maliondo (2006) mentioned that variation in the rooting ability of air layers between seasons is reported for many species; especially those that are difficult to root. This could be caused by either differences in the amount of food reserves that are stored in a plant at the season of air layering or other compounds produced by plants (Hartmann *et al*., 1990: Mwang'ingo *et al*., 2006).

The differences observed in rooting during different seasons were also described by Kathiresan & Ravikumar (1995) for root initiation on two species of mangroves in October and January (India). Rooting percentage and orientation or quadrants of the tree in this study was found to be highly correlated, since the statistical analysis showed that northern side air layers had higher rooting percentage than the other quadrants, south, east and west during spring. This can be explained by the angle of the sun rays in the southern hemisphere, hitting the tree canopy on the northern side resulting in higher temperatures on the northern and western sides of the canopy.

There are many reports mentioning that auxin application promotes rootability and root development of air layers in many woody species such as mangroves, *Osyris lanceolata,* Hazelnut, *Hymenaea courbaril*, Roseapple (*Syzigium jambos L*.), litchi and Khirni (*Manilkara hexandra* (*Roxb*) *Dub*.) (Kathiresan & Ravikumar, 1995; Thirunavoukkarasu, Brahmam & Dhal, 2004; Erdogan & Smith, 2005; Gowda, Vasanth & Shyamalamma, 2006; Mwang'ingo *et al*., 2006; Gowda,

Shyamalamma & Prakash, 2006). The opposite situation was found during this study where the best results were obtained in control treatments in spring where air layers were not treated with rooting auxin. But in autumn season, inconsistent results were obtained on control as well as on Seradix® No. 2 treatments where the IBA application had a slightly beneficial effect on rooting. In certain woody species like sweet lime, grapefruit and sour lemon, combination of NAA (1-Naphthaleneacetic acid) and IAA (Indole-3-acetic acid) at one percent produced and appreciable improvement of 95 percent could be reached in rooting of litchi, air layers but in rambutan the rooting success was variable (Reddy, 1999) which is similar to *P. capensis* rootability. Kumar, Gupta, Ahlawat & Datta (2002) achieved a high rooting percentage with an increase in growth regulator concentration in Tai neem (*Azadirachta Indica* var. *siamensis* Valenton).

This was not the same case in propagation of *P. capensis*, where control treatments produced roots four weeks earlier with a higher percentage rooting than air layers treated with Seradix® No. 2. According to Evans & Blazich (1999) application of a root-promoting substance to the exposed wound of the shoot is sometimes beneficial but other species produce roots easily when they are forced to root while they are attached to the mother plant. In fact, they get the water, mineral salts and photosynthates from the air layers since the part of the branch above the girdle had leaves that synthesize food by photosynthesis. Again the physiological status of the stock plant prior to severance of the air layer setting may actively influence the success of rooting ability and stimulatory effect of auxin (Leakey *et al*., 1982; Ofori *et al*., 1996).

During autumn, *P. capensis* trees at the Experimental Farm of University of Pretoria were flowering and most of their carbohydrates were used for metabolic activities (Kathiresan & Ravikumar, 1995). This also was assumed to be the great factor of poor rooting of air layers. Eganathan *et al*. (2000) also found that seasonal variation might be the cause of unsuccessful rooting in mangrove species as was found in *P. capensis* air layers in this study. .

3.6 Conclusion

In conclusion, the results obtained from this study indicate that rooting of *Pappea capensis* air layers was successful in spring season because very high rooting percentages were obtained, but root production was mostly affected by the mother tree on which the air layers were made, the time of the year when the air layers were prepared as well as the position of the air layer on the tree canopy. The action of the Seradix® No. 2 was inconsistent. On the northern side the control treatment produced the most and longest roots, while on the southern side the air layers treated with Seradix® No. 2 produced more, but shorter roots. Based on the results obtained in this chapter, air layering technique is very promising way of multiplying *Pappea capensis* vegetatively. On the other hand, it is a relatively cumbersome and expensive method of propagation.

3.7 References

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CHAPTER 4

INFLUENCE OF PHENOLICS AND CARBOHYDRATES ON ROOTING OF STEM CUTTINGS AND AIR LAYERS OF *PAPPEA CAPENSIS*

4.1 Abstract

Differences in root response were noticed in rooting of stem cuttings and air-layers during propagation of *Pappea capensis* (Chapters 2 and 3). Therefore, the objective of this study was to determine if total phenolic and carbohydrate contents in *Pappea capensis* cuttings and air layers were involved in rooting response. Cuttings and air layers were freeze dried for 7 days. Rooted, callused and none rooted cuttings and air layers were selected and ground with a grinder into fine powder. The concentration of phenolic compounds was determined using the Folin-Ciocalteau reagent. The supernatant was then injected into a high performance liquid chromatography (HPLC) to determine soluble sugars. The remaining pellets were used for starch analysis. Higher accumulation of phenols was noticed from callused stem cuttings and air layers. Starch results showed a correlation between rooting and auxin application on different gender trees. Therefore rooting of *Pappea capensis* stem cuttings and air layers were positively influenced by total phenolics and starch accumulation.

Keywords: *Pappea capensis*, total phenolics, soluble sugars, starch

4.2 Introduction

According to Hartmann *et al*. (2000) many tree and shrub species are clonally propagated by stem cuttings. However, there are cases where cuttings of certain species remain difficult to initiate adventitious roots and then other propagation techniques like air-layering and tissue culture are required. With clonal propagation it has been found that indole butyric acid (IBA) stimulates root formation of cuttings as it promotes root initiation and also its weak phytotoxicity and great stability characteristics relates well with other rooting substances (Hartmann *et al*., 2000).

Qaddoury & Amssa (2004) noticed that during rooting many physiological changes in enzyme activities, which regulate different biochemical pathways, take place inside the tissues. According to Osterc, Stefancic, Solar & Stampar (2008), many hydroxycinnamic and chlorogenic and ellagic acids accumulated in the cutting bases of two clones of hybrid Chestnut (Castanea crenata x Castanea sativa) during propagation, some of which improved root formation but others inhibited root initiation of cuttings. In *Pelargonium*, adventitious root formation was reported to be related to initial carbohydrate reserves and current photosynthesis (Rapaka *et al*., 2005). *Pappea capensis* was found to be difficult to root with stem cuttings. However, the air-layering technique was successful but results showed variation between the two trees indentified as two different gender types (chapter 5). Considering these facts, it was deemed necessary to analyze cuttings and air-layers for phenolic and carbohydrate contents to explain the physiological changes during vegetative propagation of *Pappea capensis*.

4.3 Materials and methods

4.3.1 Sample preparation

After rooting experiments (Chapters 2 and 3) samples were not discarded, but they were used for chemical analysis. Five cuttings and five air layers were each selected from three categories of rooted, callused and non-rooted cuttings. Samples were freeze dried with an Edwards® Modulyo Freeze Drier for 7 days to prevent oxidation of cuttings and air layers. Those that had rooted callused and those that did not produce roots or callus were ground separately into a fine powder with a grinder (IKA A11 basic). Samples (0.05 g) were weighed and placed into Eppendorf tubes for phenol extraction (Mng'omba, 2007; Wu, 2007).

4.3.2 Extraction and quantitative determination of total phenolic compounds with Folin-Ciocalteau reagent

A cold mixture (1 mℓ) of methanol: acetone: water (7:7:1, v: v: v) was added to the sample for extraction of phenolics. Each (0.05 g) sample was placed in VWR™ (USC900TH, VWR International bvba/spri, B-3001 Leuven, USC900TH, Malysia) ultrasonic water bath for 5 minutes and centrifuged at 2400 rpm for four minutes with a bench centrifuge (Combi-spin, type: FVL-2400N, Rochelle Chemicals & Laboratory Equipment, Germany). The extraction procedure was repeated twice. The supernatants were mixed and decanted into Eppendorf tubes.

The concentration of phenolic compounds was determined using the Folin-Ciocalteau reagent (Sigma) based on the reduction of phospho-molybdene/phosphor-tungstate (de Ascensao & Dubery, 2003). Deionised water (135 µℓ) was dispensed in 96-well ELISA plates for quantification of phenols, followed by sample extract (5 µℓ), and Folin-Ciocalteau reagent (20 µℓ). Sodium carbonate (40 µℓ of 20% w/v) was added and a blank in which water replaced the

sample was used as a control. Afterwards, three wells (replicates) were used per sample, incubated at 40ºC for 30 minutes. Absorbance was read at 690 nm using an ELISA reader (Multiskan Ascent VI.24354 – 50973 (version 1.3.1)). The phenolic concentration in the extracts was calculated from a standard curve (y =0.0037x) passing through the origin and expressed as gallic acid equivalent (GAE) per gram of dry mass of the sample. Gallic equivalents were calculated by the following formula:

C = cV / m (Miliauskas, Venskutonis & van Beek, 2004).

Where:

C = total content of phenols (mg/g plant extract in GAE)

c = concentration of gallic acid from the calibrated curve (mg/mℓ)

V = Volume of extract (mℓ)

m = the mass of pure plant extract (g)

4.3.3 Quantitative determination of soluble sugars and starch content

4.3.3.1 Sample preparation

The preparation of samples to determine starch content was the same as 4.3.1 (Determination of total phenolic compounds) described above.

4.3.3.2 Extraction and determination of soluble sugars

The extraction and determination procedure of sugars was adapted from Ulger, Sonmez, Karkacier, Ertoy, Akdersir & Aksu (2004); Wong, Baggett & Rye (2003) and Liu, Robinson, Madore, Witney & Arpaia (1999). Stem cuttings and air-layers from each treatment were sampled and ground using a grinder (IKA A11 basic). Each ground dried sample (0.1 g) was added to 4 mℓ 80% ethanol in Eppendorf tubes and incubated in 80ºC water bath (Julabo V LaboTec (PTY) Ltd. No.101, USA) for 30 minutes to extract ethanol soluble sugars (glucose, sucrose and fructose) and each sample was divided into three Eppendorf tubes, each

representing a replication. The extract was centrifuged for 5 minutes using a Kubota® 2010 centrifuge at 3000 rpm to pellet the tissue. The ethanol was decanted and the above procedure was repeated three times.

The ethanol extract was dried and the dried samples were mixed with 4 mℓ water. The homogenised mixture (4024.8g) was centrifuged at 6000 rpm for 30 minutes at ambient temperature with a bench centrifuge (Kubota® 2010 centrifuge). The supernatant was filtered through Cameo 30R 0.45 µm 30 mm (DD04T30LP) nylon filters. An aliquot filtrate (2.5 mℓ) was blended with acetonitrile (5 mℓ). The mixture was filtered through Cameo 30R 0.45µ 30 mm (DD04T30LP) nylon filters before 200 µℓ injections.

The sugar composition was analysed by HPLC-RP (Hewlett Packard Agilent 1100 series) with DAD detection (diode array detector, 280, 325, 340 nm), Luna 3µ C-18 (PHenomenex®) reverse phase column. Exactly 20 µl of the extract was injected and analysed by HPLC using a Sugar-pak (Waters, Milford and Mass) column. The separated sugars were detected with a 156 Refractive Index Detector and quantified by comparison to known sugar standards.

4.3.3.4 Extraction and quantification of starch

The remaining pellets were used for starch or insoluble sugar analysis. The pellets were air dried in order for ethanol to evaporate. Sodium acetate 0.025 M buffer solution (pH 4.5; 2.5 mℓ) and 50 µℓ amyloglucosidase solution (LAB CHEM (Pty) Ltd) was added and incubated in the 50ºC water bath (Julabo V LaboTec (PTY) Ltd. No.101, USA) for 20 hours to break down starch to glucose (Funk, Jones & Lerdau, 1999; Wu, 2007). The mixture test tubes were centrifuged by a bench centrifuge (Kubota® 2010 centrifuge) for 10 minutes at a speed of 3000 rpm. The supernatant (2 mℓ) was decanted into other test tubes and 2 ml σ-toluidine reagent (LAB CHEM (Pty) Ltd) was added to the test tubes containing the supernatant samples. Then they were incubated in a 90ºC water bath for 10

minutes. After that the test tubes were cooled down for 30 minutes. The samples were then poured into cuvettes and placed in a Beckman Spectrophotometer, Coulter™DU®530UV/V_{is} (Beckman Instruments, Inc. Fullerton, CA USA). The cuvettes were rinsed after every sample. The absorbance values were read at 690 nm and the percentage starch was calculated using the following formula:

Dilution Factor = 2.5 + 2 (0.05)/ 2

= 1.3

The above dilution factor calculation depends on expected starch concentration. Therefore the concentration of dry material was 0.1 g / (2.5 + (50 µℓ)) $mℓ^{-1}$.

$$\%\ \textbf{STARCH} = \frac{\textbf{C} \times \textbf{D} \times \textbf{K}}{\textbf{W}} \times \textbf{100}$$ **(**Funk, Jones & Lerdau, 1999)

Where,

C = concentration of glucose sub-sampled for colour development (Reading from spectrophotometer)

D = dilution factor of 1.3

K = water hydrolysis constant (0.9)

W = total dry mass of the sample (g)

4.3.3.4 Data analysis

Data were analysed using the PROC GLM (General Linear Models) procedure in the SAS (Statistical Analysis System version 9.1) (Carry, 2002). ANOVA (Analysis of Variance) was done to determine significant differences of the means.

4.4 Results

4.4.1 Quantitative determination of total phenolics of stem cuttings and air layers

Very high levels of total phenol compounds (17.81 mg/g) were found in stem cuttings obtained from Tree No. 2 compared to the stem cuttings (6.78 mg/g) on Tree No. 1. Table 4.1 shows the total phenolic concentrations of rooted, callused, or stem cuttings that did not root or form callus. The highest total phenolic contents were found in stems that did not root or produce callus. However, the levels of total phenolics of stem cuttings treated with Seradix® No. 2 and those not treated with IBA were similar (Table 4.1). Very high phenol contents were observed from stems treated with IBA collected from Tree No. 2 and very low amounts were obtained from all rooted cuttings of Tree No. 1 and Tree No. 2 without Seradix® No. 2 treatment.

As shown in Table 4.1, total phenolic contents obtained from air-layers collected from the two trees did not show any statistically significant differences. Total phenolics from air layers taken from Tree No. 1 and Tree No. 2 were 28.22 mg g^{-1} and 21.8 mg g^{-1}, respectively. Very high amounts of total phenolics were obtained from Tree No. 2 air layers that produced callus (34.55 mg/g) than those which rooted, and on air layers did not produce roots. Furthermore, application of Seradix® No. 2 did not significantly influence the phenol content of *Pappea capensis* air layers on both treatments.

Table 4.1 Total phenolics of rooted, callused and non-rooted stem cuttings and air layers of *Pappea capensis*

Total phenolics in stem cuttings (mg/g) CV(%)= 14.3						
Control				Seradix® No. 2		
	Rooted	Callus	None	Rooted	Callus	None
Tree No. 1	0e	12.13c	6.76d	0e	14.75c	7.09d
Tree No. 2	13.18c	27.13a	26.41a	0e	19.90b	20.25b
Average	6.59	19.63	16.58	0	17.32	13.67
Total phenolics in air layers (mg/g) CV(%)=10.1						
Control				Seradix® No. 2		
	Rooted	callus	None	Rooted	Callus	None
Tree No. 1	23.18bc	21.70bc	15.85c	23.57b	23.76b	16.88bc
Tree No. 2	17.56bc	34.55a	16.75bc	18.59bc	22.85bc	21.70bc
Average	20.37	28.13	16.30	21.08	23.31	19.29

Means within a column and treatment with similar alphabetical letters are not significantly different from each other at P<0.05.

4.2 Quantification of soluble sugars in cuttings

No soluble sugars were detected with HPLC from the stem cuttings and air-layers of the two *Pappea capensis* trees after rooting.

4.4.3 Quantification of starch in stem cuttings and air-layers

The amount of starch found from Tree No. 1 and Tree No. 2 was similar (Table 4.2). In the control, higher differences were noticed between callused, non-rooted and on rooted stem cuttings, where higher accumulation of starch was found in callused (12.89 mg g^{-1}) and non-rooted cuttings (9.23 mg g^{-1}) than in rooted (6.14 mg g^{-1}) stem cuttings of *Pappea capensis* trees. Similar variations in starch contents were observed in stem cuttings that were treated with Seradix. Rooted

stem cuttings showed much higher amounts of starch (17.24 mg g^{-1}) than callused (12.46 mg g^{-1}) and non-rooted stem cuttings (8.62 mg g^{-1}).

As indicated in Table 4.2, Tree No. 1 air layers had high starch contents than air layers taken from Tree No. 2. The starch contents of rooted, callused and non-rooted air layers were significantly different. Higher amounts of starch were found on callused air layers obtained from Tree No. 1, where Seradix was applied than on rooted air layers of Tree No. 1 (control). However, rooted air layers from Tree No. 1 under control treatment had slightly higher starch content than the other two. The same situation was found in Seradix® No. 2 treatment, even though there were no significant differences, air layers treated with Seradix® No. 2 tended to have higher starch percentage than where Seradix® No. 2 was not applied (control).

Table 4.2 Total starch percentage (%) of *Pappea capensis* stem cuttings and air layers from different trees with and without Seradix® No. 2

	Total starch in stem cuttings (mg/g) CV (%) = 21.24					
	Control			Seradix® No. 2		
	Rooted	Callus	None	Rooted	Callus	None
Tree No. 1	18.45a	14.99ab	0d	14.66ab	16.19ab	0d
Tree No. 2	0d	10.78b	12.29b	19.82a	8.73c	17.23a
Average	9.23	12.89	6.14	17.24	12.46	8.62
Total starch in air layers (mg/g) CV (%) = 18.45						
	Control			Seradix® No. 2		
	Rooted	Callus	None	Rooted	Callus	None
Tree No. 1	17.03a	7.41cd	14.67b	15.52b	20.99a	13.64b
Tree No. 2	4.29d	8.91cd	3.23d	4.15d	8.23cd	12.91c
Average	10.65	8.16	8.95	9.84	14.61	13.28

Means within a column and treatment with similar alphabetical letters are not significantly different from each other at $P<0.05$.

4.5 Discussion

According to Rapaka *et al*. (2008) adventitious root formation on stem cuttings involves many physiological changes and mostly relies on nutrients and interactions between endogenous chemicals such as phenols and carbohydrates. In this study, total phenolic content was found to be very low in rooted stem cuttings. In grape rootstock cuttings, phenolic acids were found to stimulate formation of adventitious roots (Bartolini *et al*., 1988), but high accumulation of phenols inhibited root formation in *Chamaelaucium* cuttings (Currir *et al*., 1993). Similarly, with *Pappea capensis* in this study callused stem cuttings, and stem cuttings that did not produce roots, were found to have high total phenol contents.

According to Cameron & Thomson (1969) another factor that might inhibit or promote rooting is high accumulation of total phenols at the bases of girdled shoots. A significant amount of total phenols in *Pappea capensis* air layers was found in callused, rooted and non-rooted air layers. The idea of callus production is similar to graft establishment, once the shoot is wounded, callus cells play an important role to heal the wound and give protection to the wounded part and some phenolic compounds have a great influence in cell differentiation to form roots (Errea, 1998). In *Pappea cappensis* callus formation was clearly observed on air layers after girdling the tree branches.

Haissig (1986) and Veierskov (1988) mentioned that adventitious root formation in stem cuttings relies on enough supply of available carbohydrates to the region of root regeneration; its major role is to promote root initiation and development. The soluble sugars (glucose, fructose and sucrose) were not detected with HPLC from stem cuttings of *Pappea capensis* obtained from the two trees. It was assumed that the amounts of these soluble sugars were minute such that HPLC could not detect them. Starch was depleted during rooting, and that is why callused and non-rooted cuttings had high amount of starch compared with

rooted stem cuttings. This is similar to the results found from the analysis of stem cuttings of portulaca (*Portulaca grandiflora*), where Rapaka *et al.* (2007) reported that lower rooting response in cuttings was thought to be caused by insufficient carbohydrate availability since stem cuttings do not manufacture carbohydrates.

Stem cuttings utilize stored carbohydrates from the leaves. In the case of *Pappea capensis* stem cuttings, most of the leaves fell off after two to three weeks, and this decreased the storage of carbohydrates for the rooting of stem cuttings. During leaf abscission, stored carbohydrates in the leaves were also lost but a high content of starch was obtained in stem cuttings that did not produce roots because it was not used for root initiation. Some of the carbohydrate reserves were presumably also used for the development of the new leaves on the cuttings, further reducing availability of carbohydrates for root production. In *Pelargonium,* Rapaka, Bessler, Schreiner & Druege (2005) reported that adventitious root formation was related to carbohydrates stored in cuttings and also current photosynthesis. That is why in some studies auxins applied to promote production of adventitious roots by enhancing carbohydrate transportation from the sink to where the roots get initiated (Hartmann *et al*., 1990). Even though there were no significant differences in starch content between auxin treatment and the control in both cuttings and air-layers of *Pappea capensis,* the Seradix treated cuttings and air layers were found to have higher amounts of starch than the control.

4.6 Conclusion

In conclusion, total phenolic contents significantly differed in cuttings obtained from the two different trees, and whether they were rooted, callused or non-rooted. Significantly higher contents of total phenols were detected in untreated in callused stem cuttings than in rooted stem cuttings and cuttings that did not

produce roots. Callused air layers of Tree No. 2 produced significantly higher amounts of phenolic compounds than rooted and none rooted air layers.

In both stem cuttings air layers analysed, no soluble sugars (glucose, fructose and sucrose) were detected by HPLC but starch content of cuttings and air layers were detected by a spectrophotometer.

Significant differences in starch content were only found in air layers cut from the two trees, where higher amounts were detected in Tree No. 1 than Tree No. 2. Rooted cuttings seem to have used the starch during root formation and that is why high starch contents were observed on callused cuttings and on cuttings that did not produce roots or callus. Starch was not significantly different in rooted, callused and non-rooted air layers because the air layers utilised stored starch from the mother plant. No significant differences were obtained in starch contents of *Pappea capensis* cuttings and air layers where rooting substance was applied. Therefore, the variations in starch and phenolic content of different trees lead to the investigation of gender observations (Chapter 5) of the two trees (Tree No. 1 and No. 2), which closely correlated with rooting of *Pappea capensis*.

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CHAPTER 5

INFLUENCE OF GENDER ON ROOTING OF *PAPPEA CAPENSIS* TREES

5.1 Abstract

In the literature there is no consensus whether *Pappea capensis* trees (genets) are monoecious or dioecious. The objective of the study was to observe two trees growing on the Experimental Farm of the University of Pretoria over an extended period in an attempt to solve the problem of variation in propagation of *Pappea capensis* by stem cuttings and air layering techniques since tree gender may play a role in the rooting and phytochemicals of cuttings and air layers (Chapters 2, 3 and 4 of this study). Inflorescences were collected at random in four quadrants from the two trees. Fresh flowers were dissected and examined microscopically in the laboratory and flowers were counted. Based on these results it was found that both trees are hermaphrodites but one tree (Tree No. 1) produced predominantly male flowers, while the second tree (Tree No. 2) started with male flowers but produced female flowers towards the end of the flowering period.

Keywords: *Pappea capensis*, gender, rooting, monoecious, dioecious

5.2 Introduction

In his book on plant breeding systems, Richards (1986) refers to plants derived from seed as **genets** while plants derived from cuttings are called **ramets** and since all known *Pappea capensis* trees are derived from seeds, they will be treated as genets in this chapter. According to Richards (1986), flowering plants can either be hermaphrodite or diclinous. Hermaphrodite species and diclinous species can further be subdivided as shown in Table 5.1.

Table 5.1 Classification of flowering plant genets based on spatial separation of androecium and gynoecium (Adapted from Richards, 1986)

HERMAPHRODITE	
Category	Spatial separation of androecium and gynoecium
True hermaphrodites with all flowers hermaphrodite	Stamens and ovaries in the same flower on same plant
Monoecious with all flowers unisexual	Stamens and ovaries in separate flowers on same plant
Gynomonoecious	Hermaphrodite and female flowers on same plant
Andromonoecious	Hermaphrodite and male flowers on the same plant
DICLINOUS	
Category	Spatial separation of androecium and gynoecium
Dioecious	Male flowers on one plant and female flowers on another plant
Sub-gynoecious	Male flowers on one plant and hermaphrodite and female flowers on another plant
Sub-androecious	Female flowers on one plant and hermaphrodite and male flowers on another plant
Gynodioecious	Hermaphrodite flowers on one plant and female flowers on another plant
Androdioecious	Hermaphrodite flowers on one plant and male flowers on another plant
Polygamous (including trioecious)	Some plants with male flowers, some with female flowers, some with hermaphrodite flowers, some with male and hermaphrodite flowers and some with female and hermaphrodite flowers. (Not all may occur)

Many authors (Phillips, 1951; Dyer, 1975; Palmer & Pitman, 1972; Van Wyk, 1984) reported *Pappea capensis* (Jacket plum) trees as dioecious plants where

male and female flowers are borne on separate trees. However Fivaz & Robbertse (1993) found that the trees were actually monoecious with separate male and female flowers occurring on the same tree but spatially and temporarily separated, even on the same inflorescence.

In Chapters 3 and 4 it was mentioned that cuttings obtained from two *Pappea capensis* trees growing on the Experimental Farm of the University of Pretoria were used for experiments with the rooting of stem cuttings and air layers.. Unexpected differences were found between the rooting of cuttings and air layers on the two trees. And the question was raised whether gender differences between the two trees could have played a role in the rooting potential of stem cuttings and air layers.

The main objective of this study was to determine the gender of the two trees (Tree No. 1 and No. 2) in order to supply an explanation for the differences in the rooting potential of cuttings and air layers obtained from these trees.

5.3 Materials and methods

Two mature trees (±30 years old) (Cillie, 2006) that were planted about 6 metres apart at the Experimental Farm of the University of Pretoria were used for propagation of *Pappea capensis and* collection of inflorescences. According to superficial observations, Tree No. 1 was bearing mainly male flowers while Tree No. 2 regularly produced both male and female flowers.

From July 2007 the trees were observed on a weekly basis for signs of flowering and also flower type. During 2007 the trees flowered in February and collection of inflorescences commenced in March 2007. Four inflorescences were collected at random from the four quadrants (south, east, west and north) of the trees, starting from March 2007 to May 2007.

Inflorescences continued to produce flowers over a period of four months. The date, number and sex of individual flowers occurring on the inflorescence over this period were recorded and the number of female to male flowers produced on each inflorescence was calculated.

Data collected were analysed using the PROC GLM (General Linear Models) procedure in the SAS (Statistical Analysis System version 9.1). ANOVA (Analysis of Variance) was done to determine significant differences in the mean number of flowers (Cary, 2002).

5.4 Results

5.4.1 *Pappea capensis* flowers

It was observed that *Pappea capensis* flowering is influenced by environmental conditions. In 2007, flowering started in February and then the trees stopped flowering in May when temperatures dropped (9 – 16^{o}C), but in 2008 flowering started in December. At the beginning of flowering, only male flowers were observed on both trees and female flowers were noticed towards the end of March when inflorescences bearing male flowers started producing female flowers at the bases of the spent male flowers. Figure 5.1 illustrates male and male-female flowers of *Pappea capensis* in autumn. Only two types (male and female) of flowers (male and female) were produced on the same tree. Tree No. 2 was found to produce a very high population of male flowers only in 2006 although a few female flowers were found on some inflorescences in 2008. New flower buds were continuously produced at the pedicel bases of the older flower buds (Fig 5.1 A and B). After producing male flowers from December 2006 to March 2007, Tree No. 1 started producing few numbers of female flowers from the pedicel bases of spent male flowers.

Both female and male flowers were borne on the same inflorescences and that at the beginning of flowering, the inflorescences were found to have only male flowers but as the temperatures dropped down to 15^{o}C (daily mean) at the end of March and probably due to shorter day length more female flowers were noticed. It was observed that the male flowers have eight or more stamens with yellow anthers (Fig 5.1 A) and a rudimentary pistil. The female flowers had a three-lobed ovary and a number of sterile anthers (Fig. 5.1 C).

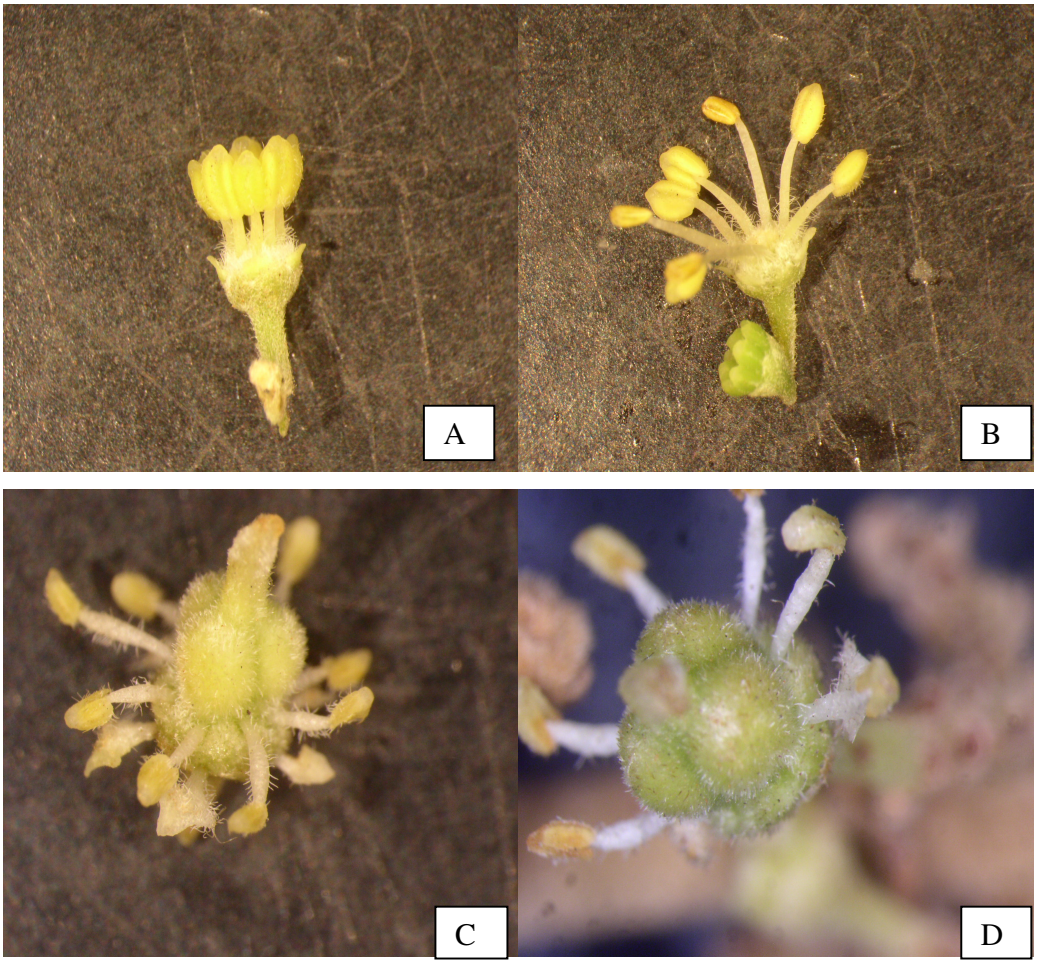

Fig 5.1 Fresh *Pappea capensis* flowers collected from Tree No. 1 (A) young male flower, (B) mature male flower with young male flower developed from the bud, (C) female flower with many anthers and (D) female flower with eight anthers

Table 5.2 shows the number of female and male flowers counted on each inflorescence in March, April and in May months. A large number of male flowers (88.75 per inflorescence) were produced by Tree No. 2 December 2006 to March 2007 and no female flowers were noticed at that time. A similar situation was observed on the other tree where very a high percentage of male flowers were observed. In April, only male flowers were produced. However in May, male

flower production decreased significantly to an average of 1.31 per inflorescence on Tree No. 1 and 22.63 on Tree No. 2.

Table 5.2 Flower percentage counted each month on selected inflorescences obtained from two *Pappea capensis* trees during a period of 3 months

		Flower percentage per inflorescence	
Tree	Month	Male	Female
No. 1	March	79.38b	0h
	April	58.31c	0h
	May	1.31g	12.69f
No. 2	March	88.75a	0h
	April	46.81d	0h
	May	22.63e	0.19h

Figures followed by the same letter are not significantly different (P>0.05), using Tukey's comparison test. Statistical comparison of each tree LSD $_{0.05}$ = 4.44. CV(%) = 8.12

5.4.3 Effect of tree type and orientation on flower production

There were no significant differences in flower production counted from inflorescences collected from four quadrants on both trees (Table 5.3). Though, inflorescences sampled from the western side of Tree No. 1 were found to have many flowers (22.92) when compared to the other quadrants for the two trees, and few flowers were counted from the eastern side (17.29) of Tree No. 1 (Table 5.3). Inflorescences obtained from the northern side of Tree No. 2 had many male flowers (43.42) than inflorescences obtained from Tree No. 1 (31.83). The highest number of female flowers (16.67) was found on the inflorescences collected from Tree No. 1 especially from the southern side of the tree.

Table 5.3: Mean number of flowers from individual inflorescences from Tree No. 1 and Tree No. 2 obtained from four quadrants (south, east, west and north) in 2007

Quadrants	South	East	West	North	Average
Tree No. 1	20.54a	21.96a	22.92a	22.13a	24.33
Tree No. 2	20.13a	17.29a	21.63a	18.13a	19.29
Average	20.34	14.68	22.23	20.13	

Figures followed by the same letter are not significantly different (P>0.05) from each other, using Tukey's comparison test. Statistical comparison was within columns of the same season. LSD $_{0.05}$ = 8.21, CV (%)= 19.23

5.5 Discussion

The results of this study showed that *Pappea capensis* trees bear male and female flowers on the same inflorescence. Inflorescences on Tree No. 1 start with male flowers first, followed by relatively few female flowers on the same inflorescence of the same tree. Similar observations were previously reported on *Pappea capensis* (Fivaz & Robbertse, 1993) and litchi trees *(*Menzel & Simpson, 1992).

However, Tree No. 2 was found to produce predominantly male flowers and very few female flowers, which sometimes cannot be noticed. Some authors reported this type of tree to be sterile (Thulin, 2001) but Tree No. 2 produced female flowers the following year and seeds were found under this tree. Seed remains were sufficient evidence that it had produced fruits in the past years. Although there was no previous study done, the flowering behaviour of this tree was assumed to be caused by environmental conditions. Even though *Pappea*

capensis can however withstand harsh climatic conditions inconsistent flower production can be the barrier for fruit production because it was selected as a potential tree to produce biodiesel in South Africa.

Menzel & Simpson (1992) reported that the excessive bearing of male flowers in mango, cashew and litchi led to reduction in yield or fruit production and this could have been another reason for the low percentage of female flowers on this tree. The age of the trees was not exactly known, but they are thought to be over thirty years old. The Tree No. 2, which is predominantly male, might be younger than the one which is more female because young trees of monoecious plants tend to be more male than female, and older trees tend to be more female (Richards, 1986).

According to this study female flower induction was affected by the seasonal change, with more male flowers that were produced from the beginning of flowering (from January to March) than in May. Richards (1986) also stated that flower gender can be affected by seasonal changes. It is possible that photoperiodic effect also played a major role to influence the floral induction of *Pappea capensis* flowers as well as the sex change of flowers. Nakata & Watanabe (1966) reported different occurrences on woody plants such as coffee, olive and litchi although floral induction in this case was associated with starch accumulation. This might also be the causative effect of floral induction in *Pappea capensis* trees. The differences in floral induction might affect root initiation by stem cuttings because both seasonal change and starch accumulation were found to influence root initiation on stem cuttings of *P. capensis*.

Hartmann *et al*. (1966) also supported the idea that carbohydrates can be a limiting factor on flower formation on trees even though sugars did not limit flower formation on olives (Ulger *et al*., 2004). As observed in Chapter 4, carbohydrates also played a major role in rooting of *Pappea capensis*. So a similar situation is

also assumed to affect the difference in tree gender of *Pappea cappensis* that is why Tree No. 2 was bearing female flowers alternatively.

5.6 Conclusion

It can be concluded that *Pappea capensis* genets are hermaphrodites, bearing separate male and female flowers on the same plant but the production of female flowers was probably affected by other factors such as temperature, nutrition and photoperiodism. It is possible that these environmental factors can also affect root initiation of stem cuttings and air layers because one tree was predominately male and the other one was dioecious. The observations made by Fivaz and Robbertse (1993) were partly accepted because the same results were found on the other tree. Therefore, tree gender seems to have an effect on rooting of both stem cuttings and air layers because higher rooting percentages were observed on layers executed on Tree No. 1 than those done on Tree No. 2. That is why there was variation in root formation and callus formation of *Pappea capensis*. Even though rooting was poor on stem cuttings, the few cuttings (5 cuttings) that rooted were obtained from Tree No. 1. Based on the results outlined in Chapters 2, 3 and 4 it is therefore a significant conclusion that tree gender had a great influence on rooting of *Pappea capensis* and also on the phytochemical contents.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

Pappea capensis belongs to the *Sapindaceae* or Litchi family. Among other indigenous trees it has selected for production of biodiesel in South Africa because of its potential for oil production. Therefore, suitable propagation methods were investigated to address the problem of rapid multiplication of the crop. The flowering study was helpful in this research because it determined whether the trees were monoecious or dioecious. This information was then applied to other aspects of the research. Various vegetative propagation experiments of *Pappea capensis* by stem cuttings and air layering were studied in spring and autumn seasons from 2006 to 2008 using the two *Pappea capensis* trees planted at the Experimental Farm of University of Pretoria in South Africa. The study analysed the physiological changes of the cuttings during rooting by determining total phenolic compounds and carbohydrates, especially soluble sugars (glucose, sucrose and fructose) and starch.

Pappea capensis trees produced male flowers which were then followed by a female phase. It was observed that *Pappea capensis* trees bear three types of flowers, viz. pistillate, staminate and hermaphrodite. But if not closely observed the above-mentioned types cannot be noticed. However, most female flowers are noticed when minimum temperatures dropped down to about 15 ^{0}C and days are shortened. One tree produced a lot of male flowers and very few female ones. That is why there was contradiction that *Pappea capensis* trees were found to be dioecious or monoecious. But the results of this study confirmed that the observations made by Fivaz and Robbertse (1993) that *Pappea capensis* tree is monoecious were correct.

According to the results obtained from stem cutting experiments, most of the stems failed to initiate adventitious roots in the spring season. However very few

stem cuttings produced rooting in autumn season and most of them callused. Survival percentage of stems treated with rooting hormone and those without hormone was not significantly different in spring, but in autumn very high survival percentage was obtained from the control. All the techniques applied in propagation of *Pappea capensis* by stem cuttings did not result in successful root initiation. Rooting was therefore thought to have been affected by physiological changes during propagation and therefore, total phenolic compounds and carbohydrates were quantified. High accumulation of total phenols was noticed from stems collected from Tree No. 2. According to the results rooted stems have lower amounts of phenols than callused stems, or the ones that failed to produce roots at all. But there was, however, no effect of auxin application. Soluble sugars in the cuttings were diminutive and nothing was detected by HPLC. The amount of starch in cuttings collected from the two trees was found not to be significantly different. Very high starch percentages were obtained from the callused and untreated cuttings. Rooted stem cuttings had very low amounts of starch. IBA application did not have any effect on rooting of stem cuttings because it did not improve root production in *Pappea capensis* stem cuttings. Therefore the unsuccessful rooting of stem cuttings made it necessary to investigate the air-layering technique as a possible alternative for vegetative propagation of *Pappea capensis* trees.

Roots were on air layers noticed after 4 weeks on both trees in spring, but in autumn roots appeared after 7 weeks. Eighty percent of air layers executed on Tree No. 1 produced roots while 32 % of air layers on Tree No. 2 produced roots in the spring season. In autumn, a different phenomenon occurred where only 48 percent and 31 percent from Tree No. 1 and Tree No. 2 rooted, respectively. In spring season high to fair rooting percentages were obtained from the north (70%), south (65%), east (50%) and west (40%) quadrants of the trees. In autumn, somewhat lower rooting percentages were observed from the north (46%), west (36%), south (41%) and east (36%) quadrants. Based on the results, application of rooting hormone (Seradix® No. 2) did not improve rooting of the air

layers because low rooting percentage were obtained in the control treatment than in the IBA treated air layers. Furthermore, it was found that the suitable season to propagate *Pappea capensis* using air layering technology is spring. On average, in spring, roots of air layers cut from Tree No. 1 were more than 60 cm long, while roots from Tree No. 2 were about 10 cm long. In autumn, roots from both trees were very short (shorter than 10 cm). Root lengths of air layers from the four quadrants were not significantly different from each other in spring and autumn seasons but very short roots were produced in autumn season. Apart from that, many more roots were counted from air layers cut from the northern side of Tree No. 1 than those from Tree No. 2 in spring especially from the control treatment. In autumn high number of roots were counted from air layers obtained from Tree No. 1, while air layers with hormone produced many roots than the control.

Physiologically, the two trees contained almost similar amounts of phenolic compounds but callused air layers had higher concentrations of total phenols than rooted and non-rooted ones. IBA treatment did not significantly influence total phenol concentrations. Similar results were obtained with analysis of soluble sugar, where nothing was detected with HPLC. However, higher amounts of starch were detected from Tree No. 1 than from Tree No. 2. Similar amounts of starch were obtained from rooted, callused and air layers that did not produce roots or callus. Starch percentages of air layers treated with IBA and those untreated were not significantly different.

Based on these results air-layering was found to be more suitable to propagate *Pappea capensis* than stem cuttings. Therefore this method can be used as an alternative vegetative propagation method for *Pappea capensis*. Again, the predominantly monoecious tree was found to be the best to propagate *Pappea capensis* especially in spring season. But further investigations must still be done with stem cuttings since these were only preliminary results. Similarly

phytochemical study and the gender issue needs much further investigation, as they relates to rooting of *Pappea capensis*.

Vegetative propagation of *Pappea capensis* Eckl. & Zeyh. (Jacket plum) by means of stem cuttings and air layers

By

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SUMMARY

Several studies have indicated the damage which fossil fuels have done to the ozone layer. Therefore, alternative ways have to be employed to reduce environmental pollution. At present some oil-rich food crops, are being selected for biofuels, including soya beans, sunflower and canola. However, alternative crops are needed to secure food supply at reasonable prices in South Africa. Some of the indigenous trees, such as *Pappea capensis* (Jacket plum), are being considered for production of biodiesel as this tree can be planted in marginal land because it can withstand the harsh climatic conditions caused by erratic rainfall.

With commercialization of biodiesel production from this undomesticated tree, high quality and quantity planting material will be required. Therefore, suitable

and rapid propagation methods need to be investigated because sexual propagated trees take many years to reach maturity and yields are unpredictable. The study objective was to investigate some suitable vegetative propagation methods as well as the influence of some phytochemicals rooting. Further limited studies on tree type and flowering were conducted to investigate possible effects on rooting of cuttings and air layers of *Pappea capensis*.

Two-well established (mature) *Pappea capensis* trees at the Experimental Farm of the University of Pretoria were used for both air layering and stem cutting experiments in spring and autumn seasons. After 8 weeks, total phenols and carbohydrates were analysed from rooted, callused and non-rooted stems and air layers.

Propagation by stem cuttings was unsuccessful because very few rooted. However, promising air layering results of 85% in control and 75% in Seradix® No. 2 treatments of Tree No. 1 were found in the spring season. On the other hand, rooting of air layers from Tree No. 1 was lower, 30% in control and 10% in IBA treatment in the autumn season. Rooting percentages of air layers of Tree No. 2 were lower than those of Tree No. 1 in both spring (60%, control and 15%, Seradix® No. 2) and autumn (5% in control) season, but higher rooting results were obtained from Tree No. 2 in autumn where air layers were treated with Seradix® No. 2. Longer roots of about 66.25 cm were produced from Tree No. 1 in spring than in autumn season (3.1 cm) from Tree No. 1. Of Tree No. 2 air layers, longer (16.55 cm) roots were observed in spring than in autumn (1.05 cm). Similarly, a higher number of roots was produced by each layer from both trees in spring than in autumn.

In stem cuttings, high amounts of phenolics were found from non-rooted (26.41 mg/g) and callused (27.13 mg/g) cuttings from the control as well as from non-rooted (20.25 mg/g) and callused (19.90 mg/g) cuttings from the IBA treatment of Tree No. 2. On the other hand, lower amounts of phenolics were found in non-

rooted (6.76 mg/g) and callused (12.13 mg/g) cuttings from the control. A similar situation of low phenolics was found on callused (14.75 mg/g) cuttings from the IBA treatment of Tree No. 1.

Analysis for soluble sugars was done with HPLC but no sugars could be detected from both cuttings and air layers. However, with starch that was analysed with a spectrophotometer, 18.45% was found in rooted stem cuttings of Tree No. 1 from the control treatment. IBA treated stem cuttings of Tree No. 2 also showed higher percentages of starch in rooted (19.82 %) and non-rooted (17.23 %) cuttings. Callused air layers from IBA treatment of Tree No. 1 had higher percentage (20.99 %) of starch than other air layers from both trees.

The two *Pappea capensis* trees were found to bear male and female flowers on the same tree, but due to unknown environmental conditions, the trees may switch from monoecious to male.

Based on results obtained air layering and stem cuttings as methods to propagate *Pappea capensis*, air layering is an alternative and suitable vegetative propagation method to obtain a high rooting percentage in the spring season, but further studies should be investigated with stem cuttings. Available phenolic compounds may influence rooting of *Pappea capensis* stem cuttings and air layers.

There appear to be very few reported investigations into the vegetative propagation of *P. capensis.* These investigations provide a starting point for further trials aimed at providing a commercially viable method of vegetative propagation. A major limitation of this study was the fact that only two trees (unnamed cultivars) provided the cuttings and air layers.