Masters in Science

"Factors affecting the successful deployment of *Pinus patula* as rooted cuttings"

Richard Glen Mitchell

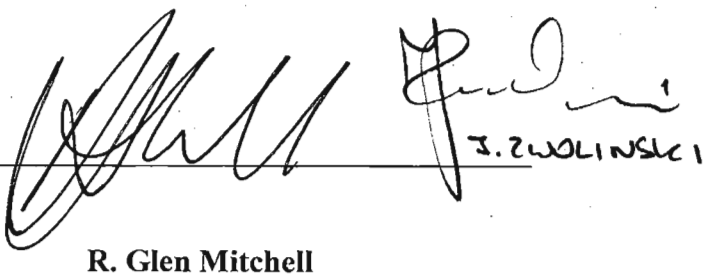
Supervisors: Prof. J. Zwolinski (UKZN)
Dr N. Jones (Sappi)
Prof. T. Coutinho (UP)

Forestry Programme
School of Agricultural Science and Agribusiness,
University of KwaZulu-Natal, Pietermaritzburg

December, 2005

Declaration

I hereby certify that the research work reported in this dissertation is the result of my own original investigation, except where acknowledged.

Signed 

R. Glen Mitchell

Table of contents

Introduction

Ensuring maximum early survival and good growth of new plantings is essential when optimizing site potential. This becomes increasingly important when improvements are made to the genetic potential of the material planted. Poor survival of improved material will negate the breeding and production efforts inputted prior to planting to improve the yield from the site.

The most important softwood species in South Africa is *Pinus patula* accounting for approximately 47% of all landholdings planted to softwoods (Bester *et al.*, 2000). Currently, the primary method of deploying *P. patula* is by seed propagated from bulk open pollinated seed mixes of superior families with an average improvement in yield of approximately 10% over material that was deployed in 1990 (Kanzler, 2004). Deployment of top elite open pollinated *P. patula* families with improved yields of as much as 30% (or greater for control pollinated families) by seedlings is restricted by the limited production of seed from these families (Kanzler, 2004). The restriction that limited seed places on the commercial deployment of high yielding planting stock is most commonly overcome through vegetative propagation of elite material as rooted cuttings. It is commonly observed, however, that when planting *P. patula* cutting material field survival is, on most occasions, poorer than seedling survival when the two plant types are planted in the same operation (Bayley and Blakeway, 2002). This observation places a severe restriction on deploying this species by means of cuttings operationally, and subsequently, on improving future yields of *P. patula*.

Local research efforts have already determined that factors such as high post planting temperatures (Morris, 1991 and Allan, 1998), the maturation feeding by *Hylastes angustatus* bark beetle (Morris, 1990), the quantity of harvesting slash around the plant (Allan and Higgs, 2000), and the effect of plant pathogens, particularly *Rhizina undulata* (Atkinson, 1997), have a significant effect on re-establishment success of *P. patula* as seedlings. It is however, not fully understood why cuttings do not achieve the same post-

planting success that seedlings do or if the factors that affect seedling survival also influence cutting survival. From limited experience when planting field trials using cutting material, it believed that the factors contributing most to poor survival and early growth after planting are, the effect of pathogens, particularly *Fusarium circinatum*, the climatic or environmental conditions during the first season after planting, the description of the planting stock at planting, and the effect of donor hedge maturation. The main aim of the series of trials carried out in this study was to identify whether these factors did affect post-planting mortality and growth, and if so, to quantify their effects.

Chapter I

The effect of applying prophylactic measures on the post-planting survival of *Pinus patula* in South Africa

Mitchell, R. G., Zwolinski, J., Jones, N. B. and Coutinho, T., 2004. The effect of applying prophylactic measures on the post-planting survival of Pinus patula in South Africa. Southern African Forestry Journal 200: 51 – 58.

ABSTRACT

The observed survival of *Pinus patula* seedlings and cuttings has, on many occasions, been inadequate in nurseries and after field planting in South Africa. There have however, been several reports that survival can be improved if a fungicide is applied at planting, indicating that mortality is partially related to pathogenic activity. This chapter summarises the results from two trials established to investigate the effects of the fungicide, Benlate (a.i. benomyl), and biological control agents, *Trichoderma harzianum* and *Bacillus subtilis*, on *P. patula* survival and growth. During the nursery raising period, the pathogen *Fusarium circinatum* was isolated from all dying material. Field results from the trials indicate that initial post-planting survival appeared to improve if the fungicide Benlate, and to some extent the biological agent *T. harzianum* was applied at planting. The apparent improvement, however, declined over time. Reasons for this are not fully explained by the trial data but it is suggested that as fungicide efficacy declines over time, young trees may become more susceptible to disease. Given the virulent nature of *F. circinatum*, it is assumed that mortality in the nursery was principally due to this pathogen, and that mortality in the field may be related to nursery infection. Due to the world wide restriction on the use of Benlate, future research should concentrate on identifying alternative fungicides and/or biological control agents that can be used in nurseries and when establishing disease susceptible species such as *P. patula*.

INTRODUCTION

It is important to optimise stand density of tree plantations to ensure effective site utilisation and economically viable timber production. This becomes increasingly important when genetically improved planting material is used at high cost. *Pinus patula* has been identified as the most important pine species planted in South Africa with approximately 47% of all softwood plantations consisting of this species (Bester *et al.*, 2000). In order to improve site productivity, the South African forestry industry is investing much of its available resources into improving the genetic potential of this species.

A number of factors may negatively influence the re-establishment of *P. patula*. These include: high post-planting temperatures (Morris, 1991), maturation feeding by the pine bark beetle, *Hylastes angustatus* (Morris, 1990 a + b), and physiological stresses due to high slash loads on the site (Allan *et al.*, 2000). It has also been observed that by applying a fungicide to the pit at planting (Allan *et al.*, 2000; Allan and Higgs, 2000; Atkinson, 1997), or as a nursery application before planting (Atkinson, 1997; Barnett *et al.*, 1988), field survival can be significantly improved. This indicates that one or more fungal pathogens are present at the time of planting.

In addition to the inadequate field survival that is often observed operationally, high mortality among *P. patula* seedlings, and particularly cuttings, has been noted in South African nurseries due, mainly, to the pathogen, *Fusarium circinatum* (Bayley and Blakeway, 2002). It is postulated that by eliminating or reducing this pathogen in nurseries, improvements in field survival, and hence in final stocking, will be achieved. Due to the increasing awareness of the dangers that fungicides pose on the environment and to the people handling such chemicals, research on alternative disease control methods, particularly biological control, is being increased. Of the biological control agents tested, *Trichoderma harzianum* and *Bacillus subtilis* have been shown to reduce the growth of some *Fusarium* species effectively (Bacon *et al.*, 2001; Muhammad and Amusa, 2003).

In this paper, the results from two trials established to test the effect of applying prophylactic measures on *P. patula* survival, are presented. The trials were designed to compare the activity of the biological control agents *T. harzianum* and *B. subtilis* as possible alternatives to the fungicide Benlate, and determine the effect of the prophylactic treatment on plant type (seedlings versus cuttings). The effect of application time of the compound applied in either the nursery or in the field, was also investigated.

METHODS

Nursery procedures

Cuttings

All cuttings set for the first trial (trial 1) were rooted and raised at the Ngodwana nursery whilst the cuttings that were set for the second trial (trial 2) were rooted at the Escarpment nursery and transferred to the Ngodwana nursery once rooted. Cutting material used in trial 1 was harvested from four families, whilst cutting material used in trial 2 was harvested from five families. The four families used in trial 1 were the same as those used in trial 2. The families included in the trials were selected for good growth. In trial 1, cutting material was set into plastic trays, containing 49 round cavities with root trainers (known as the Sappi 49 tray), and in trial 2, into plastic trays containing 98 square cavities with side-slits and root trainers (known as the Unigro 98 tray). Tray cavities were 80 ml in volume for both tray types. The growing medium used was composted pine bark in both trials.

Seedlings

The seedling material tested in both trials was obtained from seed sown operationally in the Ngodwana nursery. The seedlings were removed from production soon after germination and placed in a separate section of the nursery, along with the cuttings,

where they were raised. Due to the shorter raising period required, seed (for the seedling control) was sown in the Sappi 49 tray, in composted pine bark, approximately one month after the cuttings were set.

Treatments

Trial 1 tested the application of *T. harzianum*, *B. subtilis* and Benlate to control disease on nursery stock whilst trial 2 only tested the application of *T. harzianum* and Benlate. In both trials, plants were grouped according to treatment prior to implementation of nursery application of the prophylactics tested. Sufficient space was left between treatments to enable application of the nursery treatments and to ensure that a particular treatment was not accidentally applied to a neighbouring treatment group. The trays were rotated each time treatments were applied to eliminate edge effects.

During the nursery period products were applied weekly for 3 weeks before establishing trial 1 and every two weeks for a period of five months before establishing trial 2 **(Table 1)**. In both trials, products were applied as a nursery drench. The *T. harzianum* and *B. subtilis* treatments were applied at a rate of 450 g of product combined with 140 l of water. The supplier had suggested this rate in the absence of established recommended rates on pines in a nursery environment. The concentration of *T. harzianum* was reported to be a minimum of 10^9 fungal spores g^{-1} and for *B. subtilis*, 10^8 CFU g^{-1} (colony forming units). The Benlate treatment was applied at a rate of 1 g of product (0.5 g active ingredient) combined with 1 l of water. Products were applied to the plants using separate watering cans with rosettes (to create a spray) for each nursery treatment. All plants received standard fertilizer and water applications for the full duration of the raising period irrespective of the treatment being tested. Based on the observations of high plant mortality at the time of raising the trial material, it was hoped that sufficient inoculum existed to test the products effectively. The trials were therefore not inoculated with fungal spores.

Table 1: Treatments applied to trials 1 and 2 to test product efficacy and application timing

Treatment	Details
Control	Standard nursery practice and water planting*
Benlate – nursery	Nursery drench (1 g l^{-1}) and water planting***
Benlate – field	Standard nursery practice and water planting with 2 g l^{-1}
B. subtilis – nursery**	Nursery drench (5 g l^{-1}) and water planting***
B. subtilis – field	Standard nursery practice and water planting with 5 g l^{-1}
T. harzianum – nursery	Nursery drench (5 g l^{-1}) and water planting***
T. harzianum – field	Standard nursery practice and water planting with 5 g l^{-1}

* = Water planting is described as the application of 1 l of water over the newly established plant

** = The *B. subtilis* treatments were excluded from trial 2

*** = 3 applications in trial 1 and 10 applications in trial 2

Field planting procedures

The two trials were established over two planting seasons approximately a year apart. Site details of the two trials are summarized in **Table 2**. Trial 1 was laid out as a Randomised Complete Block design with split plots, and trial 2 as a 5x5 Latin Square with split plots. In the first trial, sub-plot treatments consisted of the four cutting families and a single seedling mix. In the second trial, subplot treatments consisted of an equal proportion of seedlings and cuttings. The five cutting families that were raised in trial 2 were mixed equally prior to planting so that each subplot consisted of precisely the same number of cuttings per family. Individual family identities were not kept.

Manual pitting was carried out on the same day and prior to planting, and the pit was, therefore, not permitted to dry out. All field treatments (**Table 2)** were applied over the plants immediately after planting and not into the pit prior to planting.

Observations

In the nursery, dying plants all appeared to show symptoms of disease by exhibiting stem and needle discolouration (purpling/browning) and early symptoms of wilt. Upon removal from the trays, roots lacked white root tips and fine root hairs, commonly observed in healthy plants. The dying plants were routinely removed and on five occasions submitted for pathological test. On two occasions plants that appeared healthy were also submitted to test for the presence of disease. This was achieved by plating pieces of plant tissue onto a selective medium, Peptone PCNB agar (Nash and Snyder, 1962, and later modified by Nelson *et al.* (1983). Fungi, which resembled *Fusarium* spp., were transferred to Spezierller Nährsstoffarmer agar (Nirenberg, 1976) and these colonies were examined microscopically and identified. At the end of the nursery raising period, all remaining plants were counted to determine the effectiveness of the disease control measures before planting.

The stem diameter and height of all plants in trial 2 were recorded the day before field planting commenced. This was done in order to determine the effect of the nursery treatment on plant size and to compare plant size in the nursery with subsequent field survival and growth.

Trees in both trials were assessed for survival at 30, 90, 180 and 360 days after planting. Growth (height, ground level diameter and biomass index (GLD^2 x Height) was calculated at 360 days.

Statistical Analysis

All data was subjected to an analysis of variance (ANOVA), using the Genstat 5, version 3.2® Statistical Programme, to determine differences between treatment means. Percentage survival data was calculated on a per plot basis and transformed using the angular transformation prior to analysis. All least significant differences (LSD) were determined at the $p<0.05$ level.

Table 2: Site and trial information for trials 1 and 2

Trial Details	Trial 1	Trial 2
Planting date	08/01/2001	01/03/2002
Location	$30^0$19"25' E; $25^0$33"10' S	$30^0$37"25' E; $25^0$37"10' S
Altitude	1700 m.a.s.l	1400 m.a.s.l
Mean annual temperature	14.8 ^{0} C	15.7 ^{0} C
Mean annual precipitation	775 mm	1174 mm
Previous species	*Eucalyptus nitens*	*Pinus elliottii*
Pre-plant herbicide spray	No	Yes
Slash burnt	Yes	No
Trial design	RCB design with split-plots	5x5 Latin Square with split-plots
Whole-plot description	Fungicide, bio-control agents or control treatment	Fungicide, bio-control agent or control treatment
Sub-plot description	4 cutting families and a single seedling mix	Plant type (cuttings or seedlings)
Number of replications	6	5
Number of treatments	7 x 5	5 x 2
Whole-plot size	35 trees	70 trees
Sub-plot size	7 trees	35 trees

RESULTS AND DISCUSSION

Nursery observations

Survival

In both trials, the pathogen, *Fusarium circinatum* was consistently isolated from all dying plant material that was analysed. No pathogenic fungi, however, were isolated from samples with a healthy appearance. From these findings it was assumed that *F. circinatum* was the main cause of mortality of the dying plants.

Although *F. circinatum* was not isolated from healthy plant material this pathogen is regularly isolated from *P. patula* plant material with a healthy appearance from nurseries in South Africa (T. A. Coutinho, pers. comm., 2003). Other studies have shown that conifer seedling roots can be infected with *F. circinatum* (Gordon *et al.*, 2000) and pathogenic strains of *F. oxysporum* (James *et al.*, 1987; 1988) without showing signs of disease. It is thus possible that in South Africa, many asymptomatic *P. patula* plants are being dispatched on a daily basis, and these undetected nursery infections could be contributing to the mortality experienced after planting.

The application of Benlate in the nursery increased survival of the cuttings compared to the control (standard nursery water and fertilizer treatment) **(Figure 1)**. The application of *T. harzianum* did not increase cutting or seedling survival. When comparing plant types, it was observed that far poorer survival occurred in the cutting material of the control and *T. harzianum* nursery treatments. Past data has shown that field survival is, on many occasions, poorer in pine cuttings compared to seedlings for reasons not fully understood (Bayley and Blakeway, 2002). It has been suggested that cuttings are more prone to disease infection as a result of the wound created when shoot material is harvested. *Fusarium circinatum* is a pathogen that is reported to require an entry port before infection can take place (Kuhlman *et al.*, 1982).

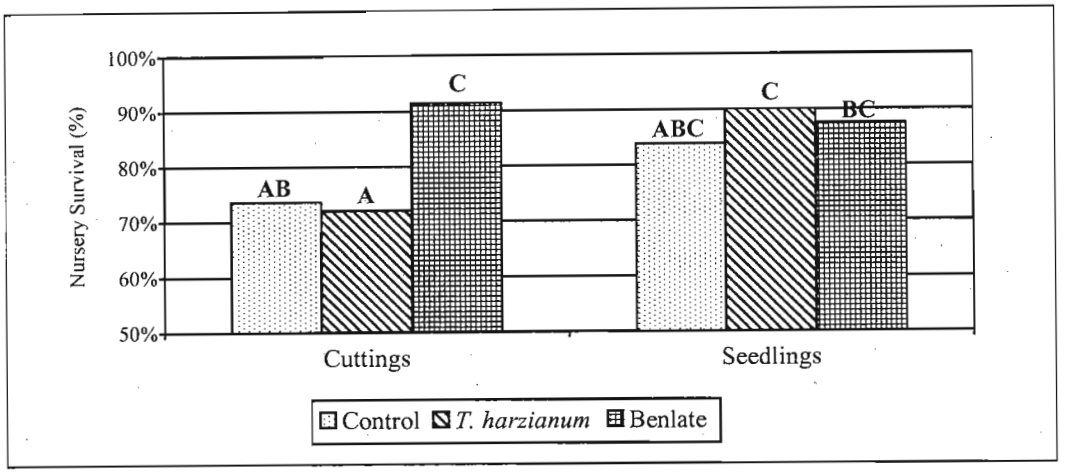


Figure 1: The effect of *T. harzianum* and Benlate applications on plant survival in the nursery in trial 2. Treatment means significantly different from one another are indicated by a different letter ($P<0.05$).

Growth

Plant dimensions, measured at the time of planting in trial 2, indicated that the application of Benlate during the nursery-raising period significantly increased plant height **(Table 3)**. This observation has been reported previously (Barnett *et al.*, 1988; Allan *et al.*, 2000) and suggests that if potential pathogenic activity can be controlled, increased resources can be allocated to growth. The application of *T. harzianum* inhibited plant growth compared to the control in this trial. It has been reported that high concentrations of *T. harzianum* may be inhibitory to plant growth (Laing and Morris, 2001) indicating that the bi-monthly rates applied may have been too frequent or that the concentration may have been too high.

Table 3: The average plant dimensions at planting (350 plants per treatment) in trial 2

	Plant size at planting				All Plants	
	Root Collar Diameter (mm)		Stem Height (mm)		Root Collar Diameter (mm)	Stem Height (mm)
Description	**Cuttings**	**Seedlings**	**Cuttings**	**Seedlings**		
Benlate	2.74	2.50	162.34	146.49	2.62	154.41
T. harzianum	2.60	2.32	137.11	93.29	2.46	115.20
Control	2.56	2.49	137.29	106.20	2.53	121.74
LSD					**0.152**	**8.805**

Field Observations

Survival

In both trials, the best survival was achieved with the application of Benlate **(Tables 4 and 5)**. In comparison to the control, however, the application of Benlate was not significant at the $p<0.05$ level, and furthermore, this response diminished over time. In the first trial the greatest response from Benlate was from field application whilst in the second trial Benlate performed equally when applied in the nursery or in the field. This may be as a result of the greater number of nursery applications during the nursery raising period for trial 2 (10 compared to 3 in trial 1) and over a longer period (5 months compared to 3 weeks).

The response to the biological control treatments was less satisfactory. The *B. subtilis*-treated plants resulted in very poor survival in trial 1 and this treatment was subsequently omitted from trial 2. In both trials, there appeared to be an initial positive response to the application of *T. harzianum* in the field compared to the control, although similarly to the Benlate treatment, this was not found to be significant at the $p<0.05$ level. In trial 2, the initial positive response to the *T. harzianum* application in the field diminished rapidly

after 180 days and this treatment ranked the poorest at 360 days. This treatment, however, remained the second best ranking treatment in trial 1. The application of *T. harzianum* in the nursery did not appear to have a positive influence on field survival, and similar to the field application, this treatment produced dissimilar results measured at 360 days in the 2 trials **(Tables 4 and 5)**. This highlights the often unpredictable response that can be obtained when working with biological agents.

Table 4: The survival percentage measured per whole-plot treatment since planting for all plants in trial 1

Whole-plot treatment	30 Days	90 Days	180 Days	360 Days
Control	85.2 *(72.6)*	67.1 *(58.1)*	61.4 *(54.1)*	57.1 *(50.1)*
Benlate in nursery	88.1 *(75.3)*	72.9 *(62.5)*	64.8 *(55.6)*	62.9 *(54.4)*
Benlate in field	91.9 *(80.3)*	78.6 *(68.2)*	70.0 *(59.7)*	64.8 *(55.5)*
B. subtilis in nursery	83.3 *(70.5)*	65.2 *(56.0)*	56.2 *(49.4)*	48.1 *(43.5)*
B. subtilis in field	88.1 *(77.0)*	68.6 *(57.4)*	61.9 *(52.3)*	57.6 *(49.7)*
T. harzianum in nursery	86.7 *(73.9)*	60.5 *(52.9)*	54.3 *(48.7)*	48.6 *(44.6)*
T. harzianum in field	90.5 *(77.3)*	76.2 *(64.1)*	69.5 *(58.9)*	63.8 *(54.4)*
LSD	***(9.277)***	***(11.14)***	***(11.65)***	***(13.00)***

(Italisised values in parenthesis represent transformed means using the angular transformation)

Table 5: The survival percentage measured per whole-plot treatment since planting for all plants in trial 2

Whole-plot treatment	30 Days	90 Days	180 Days	360 Days
Control	97.7 *(84.0)*	87.1 *(69.4)*	83.1 *(66.2)*	59.1 *(50.5)*
Benlate in nursery	97.1 *(85.0)*	93.4 *(78.5)*	91.4 *(77.3)*	60.6 *(51.4)*
Benlate in field	99.1 *(87.1)*	95.1 *(78.3)*	92.9 *(75.6)*	62.0 *(52.2)*
T. harzianum in nursery	97.7 *(82.4)*	86.3 *(68.8)*	82.3 *(65.8)*	57.4 *(49.3)*
T. harzianum in field	98.3 *(85.9)*	90.9 *(74.2)*	89.4 *(72.7)*	52.9 *(46.4)*
LSD	***(5.28)***	***(7.12)***	***(8.02)***	***(10.31)***

(Italicised values in parenthesis represent transformed means using the angular transformation)

At 360 days after planting two cutting families were found to show significantly better field survival than the seedling treatment in trial 1 **(Table 6)**. This highlights that breeding with families, observed to be more tolerant to pathogens, may be a tool in overcoming poor survival in the field in the longer term. Cutting survival was as good as seedling survival in trial 2 **(Table 7)**. The comparatively good survival of the cuttings, particularly in trial 1, is encouraging, as this phenomenon is not commonly observed on an operational scale. The reason for the better cutting survival may be as a result of better care received when handling and planting trial material. This highlights the need for further research in this area.

Table 6: The survival percentage measured per sub-plot treatment since planting across all treatments for trial 1

Sub-plot treatment	30 Days	90 Days	180 Days	360 Days
Cutting family A	87.1 (*73.8*)	66.7 (*56.4*)	58.5 (*50.3*)	54.4 *(47.5)*
Cutting family B	88.8 (*77.1*)	69.4 (*58.4*)	59.5 (*51.5*)	56.5 *(49.6)*
Cutting family C	86.7 (*74.3*)	72.4 (*61.6*)	67.7 (*57.7*)	60.5 *(52.6)*
Cutting family D	91.5 (*78.5*)	81.0 (*69.5*)	73.5 (*62.3*)	67.0 *(56.9)*
Seedlings	84.4 (*72.7*)	59.9 (*53.5*)	53.7 (*48.6*)	49.3 *(45.0)*
LSD	***(6.13)***	***(6.91)***	***(7.48)***	***(6.72)***

(Italicised values in parenthesis represent transformed means using the angular transformation)

Table 7: The survival percentage measured per sub-plot treatment since planting across all treatments for trial 2

Sub-plot treatment	30 Days	90 Days	180 Days	360 Days
Cuttings	97.7 (*84.1*)	90.5 (*73.2*)	88.1 (*71.1*)	58.9 *(50.1)*
Seedlings	98.2 (*85.7*)	90.6 (*74.5*)	87.5 (*71.9*)	57.9 *(49.8)*
LSD	***(3.83)***	***(3.59)***	***(4.50)***	***(4.43)***

(Italicised values in parenthesis represent transformed means using the angular transformation)

The positive response observed in survival when applying the fungicide in the nursery or at planting, and to some extent, applying *T. harzianum* at planting, could be due to a number of unknown factors. A current hypothesis is that the primary source of pathogenic activity is in the nursery and pathogens are suspected of being transported on asymptomatic plant material to the field at planting. If this is true, then the positive response to fungicide application either in the nursery or the field is due to disease elimination on the plant just before or at the time of planting. If, on the other hand, the primary source of pathogenic activity is in the field rather than the nursery, it can be

assumed that either the residual fungicide from nursery applications, or the direct effect of field application, is responsible for eliminating pathogens in the field and improving initial survival. Therefore, understanding where pathogens are most active, and whether asymptomatic plant material infected with *F. circinatum* is being transported to the field, is crucial to determining appropriate disease management strategies.

Growth

Conflicting growth responses were observed at 360 days in the two trials to the various treatments applied. Plants treated with Benlate, particularly at planting, ranked the largest in height, GLD and biomass index in trial 1 but not in trial 2. In trial 1, the Benlate treatments were however only significantly larger than the trees treated with *T. harzianum* in the nursery and not the control **(Table 8)**. During a routine check at approximately 270 days after planting, it was observed that trial 2 had been severely affected by accidental herbicide scorch, and it is suggested that this may have affected the integrity of the growth data in trial 2.

Table 8: Average tree size per treatment measured at 360-days since planting in trial 1

Treatment	Height (cm)	Ground Level Diameter (mm)	Biomass index (GLD^2 x Height) (cm)
Control	73.79	16.83	269.6
Benlate in nursery	77.44	17.49	297.1
Benlate in field	76.72	18.01	314.8
B. subtilis in nursery	68.77	16.08	244.3
B. subtilis in field	71.39	16.26	252.0
T. harzianum in nursery	64.48	14.45	184.4
T. harzianum in field	72.05	16.80	273.3
LSD	**10.310**	**2.231**	**76.41**

CONCLUSIONS

These trials indicate that initial post-planting survival of *P. patula* cuttings and seedlings may possibly improve by the application of prophylactic treatments highlighting that pathogenic activity is contributing to mortality. Overall survival for all treatments including the best ranked treatment (Benlate applied in-field) was unacceptably poor suggest that once the efficacy of a prophylactic treatment wears off, young trees may succumb to disease. Benlate was also observed to increase plant height in the nursery, and produced trees that ranked the largest at 360 days in the field in one trial.

The application of the biological control agents proved less successful than the Benlate treatment. The strain of *Trichoderma harzianum* tested had some effect on improving early field survival if applied at the time of planting but produced conflicting results in the two trials. The application of the *Bacillus subtilis* strain had no positive influence on field survival.

Differences in family survival in the cuttings in trial 1 allude to the possibility that a long-term solution to the problem, and a way of reducing the need for applying prophylactic measures at planting, may be attained through breeding with families more likely to achieve good survival.

Lastly, it was encouraging to note that in both trials, cutting survival was either as good as or better than seedling survival. This is not commonly observed among operational plantings and indicates that successful forest reestablishment with *P. patula* cuttings is possible. It also indicates the need for further research to be carried out in this area.

ACKNOWLEDGEMENTS

We wish to thank the following people for their contribution to these trials

i. Dr. Helga Dugatat, of Dagutat Biolab, for generously sponsoring the biological control products tested,

ii. The Escarpment and Ngodwana Nursery Managers, Mr. L. Williams and Mr. M. O'Donovan, for their assistance in providing the required plant material and nursery space, and

iii. The students and Ms Riana Jacobs, working at the diagnostic clinic of the Tree Pathology Cooperative Programme, for isolating and identifying the pathogens responsible for mortality in the nursery from the dying material submitted

Chapter II

Alternatives to Benomyl fungicide for controlling *Fusarium circinatum*: results from *in vitro* studies

ABSTRACT

Recent evidence indicates that the pathogen *Fusarium circinatum* is contributing significantly to mortality in newly established *Pinus patula* seedling and cutting stands in South Africa. In each of these studies the application of a fungicide containing the active ingredient, Benomyl, appeared to improve survival and in one study the biological control agent, *Trichoderma harzianum*, improved survival for the first 90-days after planting. Benomyl, however, has been classed as a hazardous chemical and its use in certified forest operations has been prohibited. This has necessitated determination of safer, alternative chemicals, and exploration of biological agents on controlling *F. circinatum* in the nursery and at planting.

Laboratory studies on the colony growth of two South African isolates of *F. circinatum* on half-strength potato dextrose agar (PDA) medium supplemented with nine alternative chemicals reported to control pathogens, and the biological control agent, *Trichoderma harzianum*, indicated that several chemical products and the biological agent, significantly controlled the growth rate of the pathogen, *in vitro*. Testing the effectiveness and phytotoxicity of these products *in vivo*, however, remains essential before they can be recommended for operational use.

INTRODUCTION

Since it was first identified, in 1990, in a single nursery on dying *P. patula* seedlings (Viljoen *et al.*, 1994), *F. circinatum* has become a serious threat to most pine producing nurseries in South Africa. In addition to nursery losses, recent indications suggest that this pathogen is now causing mortality in newly established seedling and cutting stock, suggesting that the problem is no longer confined to the nursery (Allan *et* al., 2000; Crous, 2004; Mitchell *et al.*, 2004b; Rolando and Little, 2004).. Moreover, plants appear free of any disease symptoms at planting indicating that they are either carrying the pathogen in a cryptic form from the nursery to the field, or that they are infected from other sources in the field. Currently, the former theory is the more popular belief amongst South African forest researchers and nurserymen and the approach in controlling this pathogen has been to combine the responsible use of chemicals whilst applying strict hygiene measures in the nursery. There is, however, insufficient knowledge on which chemicals are most suited to controlling *F. circinatum* that can simultaneously be described as being environmentally safe. Alternative control, using biological agents, has already been shown to be effective on *F. circinatum* using strains of *Arthrobacter* (Barrows-Broaddus *et al.*, 1983) and other studies indicate strong inverse relationships between the amount of naturally occurring *Trichoderma* and *Fusarium* species found on Douglas-fir seed (James *et al.*, 1987).

The aim of this study was to identify alternative chemicals to those containing the active ingredient Benomyl, and to test the effectiveness of a single strain of *Trichoderma harzianum* (strain Kd) developed by Prof. Mark Laing at the University of KwaZulu Natal, in controlling *F. circinatum*. To eliminate the unexplained variation that can occur *in vivo*, and to avoid the dangers associated with inoculating plants with *F. circinatum* in the nursery environment, it was decided to test the products under controlled conditions in the laboratory before conducting any further tests in the nursery or field.

METHODS

Materials and media

The first trial was carried out on the 14/01/2004 to test for antagonistic effects of *T. harzianum* on *F. circinatum*, and the second trial was initiated on the 30/08/2004 to test the effect of ten chemicals (including Benomyl) on controlling the rate of colony growth of *F. circinatum*. In each case, two South African *F. circinatum* strains (3577 and 3579) were used. These were provided by Forest and Agricultural Biological Institute (FABI) at the University of Pretoria, where the trials were conducted. The *F. circinatum* and *T. harzianum* isolates used in the trial were cultured on five 65 mm diameter Petri dishes containing half strength potato dextrose agar (PDA) media (24 g l^{-1} PDA, 1 g l^{-1} glucose and 1 g l^{-1} yeast extract) one week before each trial was initiated.

Biological control trial

The method developed for testing the impact of *T. harzianum* on several other plant pathogens (Bell *et.* al., 1982) was used in this study. A 5 mm diameter plug, containing expanding colonies of *T. harzianum* hyphae, was removed from the edge of the actively growing culture and placed approximately 1 cm from the edge of a separate Petri dish containing PDA medium. Similarly, using one of the *F. circinatum* strains available, a 5 mm diameter round plug was removed from the actively growing culture and placed directly opposite the *T. harzianum* sample, approximately 1 cm from the inside edge of the Petri dish. The process was then repeated on a separate plate selecting the second *F. circinatum* strain. In this way two paired cultures were created each containing one sample of a single *F. circinatum* strain and a one sample of the *T. harzianum* strain opposite one another. The trial was replicated 5 times. At the same time as the trial was initiated, a 5 mm diameter plug of each *F. circinatum* strain and the *T. harzianum* was placed onto three separate Petri dishes to compare their uninhibited growth with that of the paired cultures for visual observations only.

The paired cultures were then incubated at a constant temperature of 25 °C for a period of 7 days under 24 hour white florescent light before being assessed. It was decided to use the scoring system previously reported by Bell *et. al.* (1982) to assess the effectiveness of *T. harzianum* in controlling pathogen growth where the degree of antagonism was scored from 1 to 5, where: 1 = *T. harzianum* completely overgrew *F. circinatum;* 2 = *T. harzianum* colonized at least two-thirds of the medium surface; 3 = *T. harzianum* and *F. circinatum* each colonized approximately one half of the medium surface and neither appeared to dominate the other; 4 = *F. circinatum* colonized at least two thirds of the medium surface; and 5 = *F. circinatum* completely overgrew *T. harzianum.*

Once the paired tests had been assessed, cultures were placed in a fridge at 2 - 4 °C to prevent further growth until they were transported on the 12/02/04 to the University of KwaZulu-Natal in Pietermaritzburg to study the zone of interaction (interface region) between the *T. harzianum* and each *F. circinatum* strain using the scanning electron microscope (SEM). Specimens were prepared for viewing in the following manner; Several 5 mm diameter samples were removed from the interface region and all excess agar medium was removed from the base of each specimen without damaging the hyphae mass. Specimens then underwent chemical fixation in 3% glutaraldehyde and 0.05M sodium cacodylate buffer overnight to preserve cell structure. Once fixed, specimens were rinsed twice in 0.05M sodium cacodylate buffer and then dehydrated using the critical point drying (CPD) technique where the specimen is subjected to increasing concentrations of alcohol (30, 50, 70, 80, 90 and 100% for 10 minute durations). This was followed by immersion in liquid CO_2 (1 hour) in a CPD apparatus. Several flushes of fresh liquid CO_2 ensured that all the alcohol was replaced with the liquid CO_2. The liquid CO_2 was then gradually heated under pressure and once the critical point had been reached (74 Kgf/cm^2 and 31 °C) the CO_2 gas was very slowly removed from the apparatus. Lastly, specimens were mounted and rendered electrically conductive by means of sputter coating where a gold-palladium target was bombarded with molecules of argon gas and a fine layer of gold:palladium was released, coating the specimen (Bruton, 2003). Prepared specimens were then viewed under the SEM [(Phillips XL 30

ESEM) (high vacuum mode at 15 kV and spot size 4)] and relevant images captured for interpretation.

Chemical control trial

Ten chemicals (including Benomyl) were tested in the second trial **(Table 1)**. All of the chemicals, with the exception of Bulab® 6044 (12% sodium hypochlorite), were chosen because of their reported effect on controlling various plant pathogens and each differed in active ingredient. Bulab® 6044 is registered for use as an oxidizing microbicide to control algae and bacterial growth and is currently applied, at a rate of 1%, to sanitize implements, walkways and the growing environment in the Sappi Ngodwana nursery. It was included in this trial to test its effectiveness in this application. For the nine other chemicals tested, each can be administered as a drench, directly over plants during the growing period. In most cases, these chemicals have been applied from time to time at the Ngodwana nursery at a standard rate of 0.1% (1 g or 1 ml combined with one litre of irrigation water) with no observed detrimental effects to the plants, such as needle discolouration or stunting. It was decided, therefore, to test rates of 0.05% (half strength), 0.1% (standard strength) and 0.2% (double strength) in each of the 9 plant products, and 0.5%, 1% and 2% for the industrial grade sodium hypochlorite used.

Table 1: A description of the products and their characteristics tested in the trial

Trade name	Active ingredient (concentration)	Description	Appearance	Toxic evaluation	Cost
Benomyl 500 WP®	Benomyl (50%)	Fungicide	Powder	Described as minimally hazardous with LD_{50} of 10,000 on rats (dermal and oral). Is very toxic to fish and earthworms, however and has been listed as a possible hazard to the mammalian reproductive system. Persistence in soil of 90 – 180 days.	R67.86 – R77.51 (per kg)
Folicur EW 250®	Tebuconazole (25%)	Fungicide	Liquid	Harmful if swallowed. Irritating to skin and can cause serious damage to eyes. The toxicity on rats is 1,650 mg/kg (oral) and >5,000 mg/kg (dermal). Severely irritating to mucous membranes of rabbit (Bayer, 2000).	R151.34 – R166.34 (per litre)
Octave®	Prochloraz manganese (50%)	Fungicide	Powder	Not classified as hazardous with LD_{50} rating on rats 2,700 mg/kg (oral) and >2,000 mg/kg (dermal). May cause slight skin and eye irritation. May cause gastro-intestinal irritation and possible liver damage. Persistence of 92 to 171 days in soil (Aventis Cropscience, 2000a).	R376.13 (per kg)
Previcure - N®	Propamocarb hydrochloride (66.5%)	Fungicide	Liquid	Not classified as hazardous with LD_{50} rating on rats approximately 4,000 mg/kg (oral) and 4,500 mg/kg (dermal). Not irritating to rabbit skin with slight, reversible irritant to rabbit eyes. Persistence of 10 to 27 days in soil (Aventis Cropscience, 2000b).	R333.84
Tilt 250 EC®	Propiconazole (25%)	Fungicide	Liquid	Classified as a hazardous chemical with LD_{50} rating on rats >2,000 mg/kg (oral) and >4,000 mg/kg (dermal). May cause lung damage if swallowed and is irritating to skin and eyes. Very toxic to algae and toxic to fish and *Daphnia* (Syngenta, 2001).	R124.79 (per litre)
Citrex®	Citric acid (3%), lactic acid (3%), ascorbic acid (3%), sodium chloride (1%), ammonium hydroxide and ammonium propionate (1%)	Sterilant	Liquid	Recognized as safe. Can be used in animal feed and drinking water to control internal parasites. Large amounts ingested may be harmful. No harmful effects to skin. Contact with eyes may cause irritation. Persistence of less than 14 days in the soil (Citrex, 2000 and Venter, pers. comm., 2004).	R550.00 (per litre)
Bulab 6044®	Sodium hypochlorite (12%)	Sterilant	Liquid	Corrosive to skin, eyes the nose and throat. Very toxic to fish. Persistence is less than 5 days (Buckman Laboratories, 2000).	R4.50 (per litre)
Koppersprey®	Copperoxyichloride (50%)	Sterilant	Powder	LD_{50} rating of 1,400 mg/kg. Contact with the skin and eyes can cause irritation. Very toxic to fish and aquatic organisms.	R20.50 – R43.32 (per kg)*
Prasin Agri®	Polymetric biguanide hydrochloride and quaternary ammonium (7%)	Sterilant	Liquid	Very low toxicity in rats with a LD_{50} rating of 20,000 mg/kg (oral). Prolonged skin contact may cause irritation and may cause eye irritation. Very toxic to fish and aquatic invertebrates (Prasin Agri, 1999).	R75.50 – R85.00 (per litre)
Sporekill®	N,N – Didecyl N,N – dimethylammonium Chloride (12%)	Sterilant	Liquid	Very low toxicity with a LD_{50} rating of >4,000 mg/kg (oral) in rats. Contact with the skin may produce slight irritation and direct eye contact will cause severe irritation. May cause burning pain in the mouth, throat and abdomen (Hygrotech, 2000).	R61.53 – R64.71 (per litre)

While the PDA was still in a liquid state (± 70 °C) the exact quantity required for making up each treatment was dispensed using a Jencons Perimatic calibrated liquid dispenser and the chemical products added at the specific rates. After the products were thoroughly mixed, the medium was poured into Petri dishes and allowed to solidify at room temperature overnight. The following day a 5 mm diameter plug of *F. circinatum* culture was placed in the centre of each Petri dish containing the treated media. A single strain of the inoculum was placed in the centre of each plate containing the chemical treatment and the trial was replicated 3 times. In order to compare the efficacy of chemical treatments against a control, the two strains were also plated onto PDA media containing no added chemical and replicated 3 times. The trial was, therefore, designed as a 10 x 3 x 2 factorial (10 products x 3 rates x 2 strains) with an additional control treatment containing no chemicals.

Cultures were sealed and left to incubate for a period of 7 days at room temperature before being assessed for colony growth. Assessments were carried out by calculating the average colony development after measuring diameter growth in two opposite directions. The size of the original 5 mm plug was subtracted from the diameter assessments in order to analyse new growth only. Data was subjected to an analysis of variance (ANOVA) using the Genstat 5, version 3.2® statistical program and all least significant difference (LSD) values were calculated at the $p<0.05$ level of significance.

Due to the fact that the treated cultures were tested at 3 rates and the untreated culture was tested at the single control rate the data was unbalanced. A dummy variate (contrast) was, therefore, introduced to identify those cultures that were grown on treated media and the treated cultures were then compared with the control.

RESULTS AND DISCUSSION

Biological control

The *T. harzianum* strain, grown in the visual control, rapidly colonized the entire medium surface by day 7 **(Figure 1)** compared to the slower growing *F. circinatum* strains **(Figure 2)**. When the paired cultures were assessed the *T. harzianum* strain had occupied almost the entire surface of every Petri dish in the trial and severely restricted the growth of the two *F. circinatum* strains. Each plate was, therefore, scored a value of 2 **(Figure 3)**. In one or two cases the *T. harzianum* had started to grow over the *F. circinatum* colony but as the *F. circinatum* was not completely overgrown, a score of 1 was not given. It was therefore unnecessary to apply statistics to the data and it can be reported that the *T. harzianum* strain was visibly highly antagonistic towards *F. circinatum* on PDA medium. In the study conducted by Bell *et al.* (1982), paired cultures were left to incubate at room temperature (approximately 26 °C) for a period of 5, and not 7 days. Assessing the cultures after 5 days may have allowed for a better assessment as the growth of the cultures could probably have been more easily measured and analysed before the entire medium surface had been colonized. It is doubtful, however, that the results of the analysis would have resulted in a different conclusion.

Studying the interface region of the two fungi under SEM revealed that in areas where the *T. harzianum* hyphae dominated, the broader and somewhat darker, *F. circinatum* hyphae had collapsed and their walls were no longer clearly defined compared to the lighter, thinner, *T. harzianum* hyphae. *Fusarium circinatum* conidia (spores) were also completely absent at this region whereas *T. harzianum* conidia were found in abundance **(Figure 4)**. This indicates that *T. harzianum* not only slowed down the rate of *F. circinatum* growth, but was also able to promote its own growth despite the presence of the pathogen. After a few more days it was observed that the *T. harzianum* hyphae had completely overgrown the pathogen and that the *F. circinatum* culture was visibly disintegrating.

Figure 1. ***Trichoderma harzianum*** **(strain Kd) grown at 25 °C for 7 days on half strength PDA medium**

Figure 2. ***Fusarium circinatum*** **(strain 3577) grown at 25 °C for 7 days on half strength PDA medium**

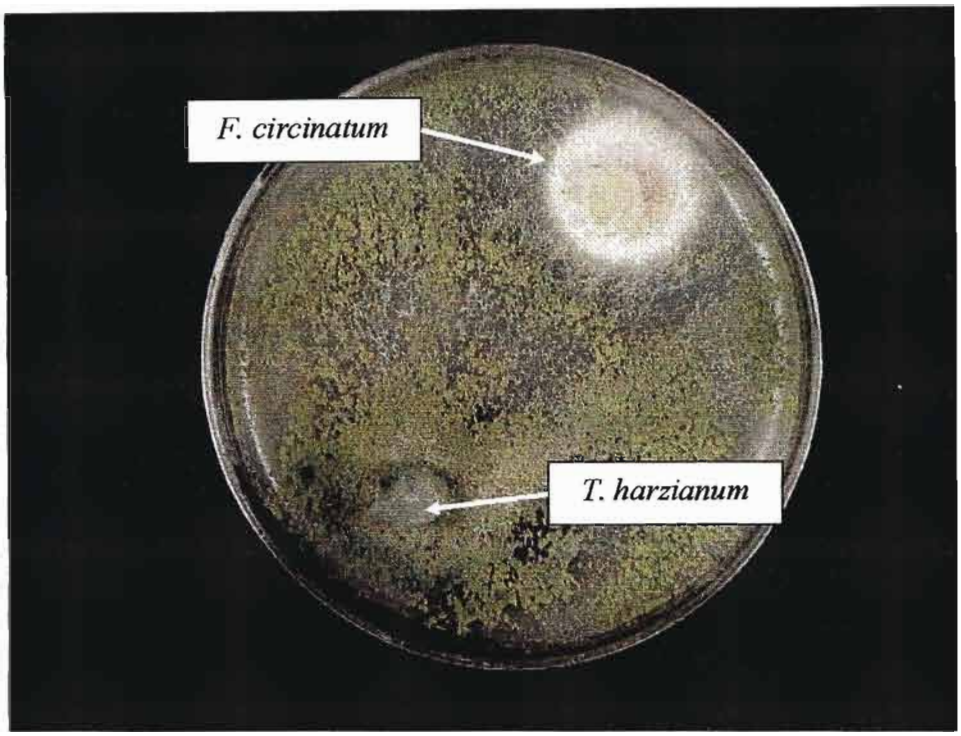


Figure 3. Paired culture of *F. circinatum* (strain 3577) and *T. harzianum* (strain Kd) grown at 25 °C for 7 days on half strength PDA medium

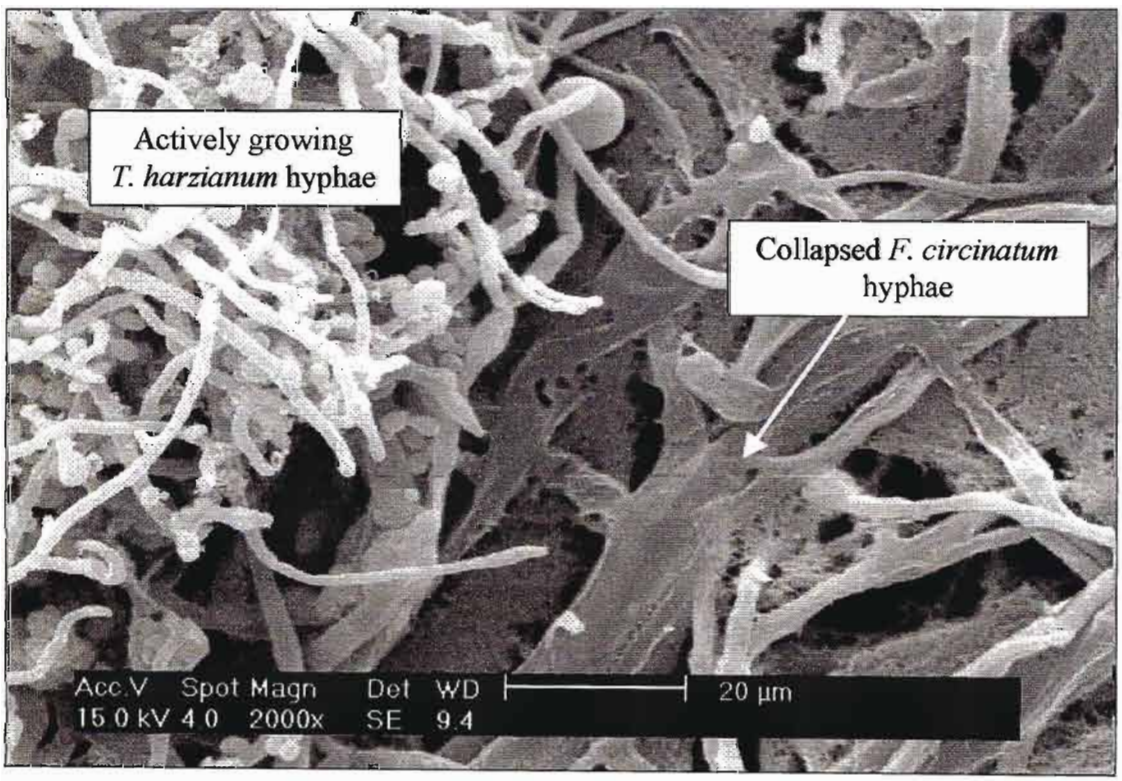


Figure 4. Interface region between *T. harzianum* and *F. circinatum* under the scanning electron microscope

Chemical control

The results of the ANOVA on the chemicals tested to control *F. circinatum, in vitro,* indicate that on average, the cultures grown on PDA medium treated with chemical products grew significantly slower than those grown on untreated PDA medium. Within the treated cultures, however, there were significant differences indicating that some products were more effective than others **(Table 2)** and that several of these were found to be as effective as Benomyl **(Figure 5)**. With each product tested, increasing concentrations brought about a further reduction in colony growth, and the mean of each application rate was found to differ significantly from the other and from the control **(Figure 6)**.

The significant interaction between product and strain, and rate and strain **(Table 2)**, highlights the importance of including several strains in such studies. For example, with the exception of one treatment in this trial, strain 3579 always colonized the medium surface faster than 3577, and by doubling the application rate, better control was achieved on the growth of 3579 with some products. It would be prudent, therefore, to determine the dominant strains inhabiting the nursery environment before conducting such trials, and to test products on several dominant strains.

Table 2. An ANOVA table showing the contrasts performed on the colony growth (diameter) data in response to chemical treatments (type and rate) of the *Fusarium circinatum* strains.

Source of variation	d. f.	s. s.	m. s.	v. r.	F pr.
Rep	2	52.88	26.44	2.15	=0.121
Contrast Untreated vs. Treated	1	5129.66	5129.66	414.71	<0.001
Contrast.Product	9	29936.52	3326.28	270.21	<0.001
Contrast.Rate	2	3349.82	1674.91	136.06	<0.001
Contrast.Strain	2	802.20	401.10	32.58	<0.001
Contrast.Product x Rate	18	4855.36	269.74	21.91	<0.001
Contrast.Product x Strain	9	594.17	66.02	5.36	<0.001
Contrast.Rate x Strain	2	95.12	47.56	3.86	=0.024
Contrast.Product x Rate x Strain	18	386.72	21.48	1.75	=0.040
Residual	122	1501.80	12.31		
Total	185	46704.25			

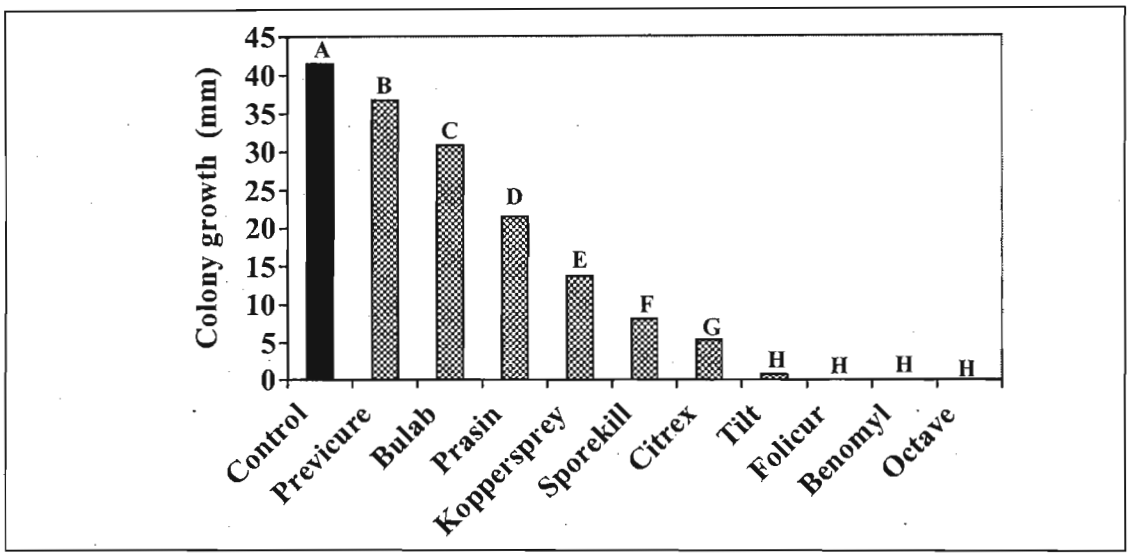


Figure 5. Mean colony growth (mm) of *F. circinatum* on PDA medium containing 10 chemical products reported to control pathogens. (Treatment means indicated by different letters are significantly different at the 5% level).

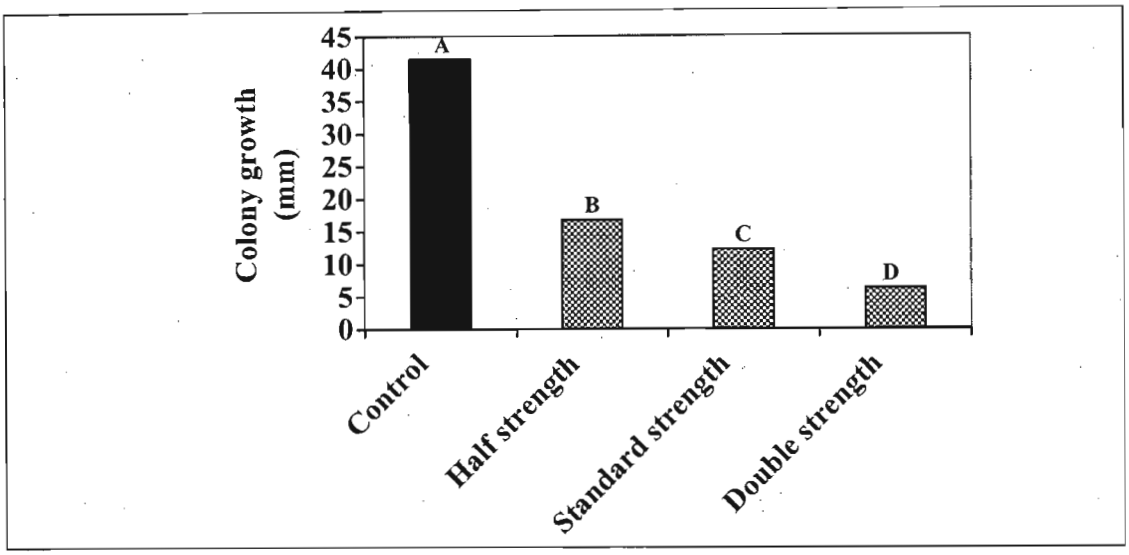


Figure 6. Mean colony growth (mm) of *F. circinatum* on PDA medium containing 10 products at half, the current, and double the standard rate applied in the nursery. (Treatment means indicated by a different letter are significantly different at the 5% level).

Fungicides

The fungicides most comparable with Benomyl in controlling *F. circinatum* in this trial were Octave® (prochloraz manganese), Folicur® (tebuconazole) and Tilt® (propiconazole). Of these three, Octave® poses the least risk to the environment **(Table 1)** and should therefore be included in future trials to determine whether phytotoxic effects exist and whether the product is effective *in vivo*. Although this product poses less danger to humans and the environment compared with the others, a report has indicated that 8-month-old trees were stunted after receiving an application of 2 g (active ingredient) of a similar prochloraz-based product (Sporgon®) per seedling at planting (Allan *et al.*, 2000). This indicates that high amounts of this chemical may be phytotoxic to young plants. Tilt, although currently still an accepted fungicide is several times more toxic than Octave®. If this product is to be tested for future use it should be done so on the understanding that it

may only be available for a limited period of time. Folicur® is less toxic than Tilt® **(Table 1)** and may prove to be a useful alternative.

Previcure® (propamocarb hydrochloride) had the least influence of all the products tested on reducing hyphae growth. This carbomate fungicide is probably the least toxic of the 4 alternative fungicides tested and has a very short half-life **(Table 1)**. Future testing of this product should be reserved for use on other nursery pathogens, and if found to be effective, its low toxicity level would favour its application. The future use of Previcure® on *F. circinatum*, however, should not be explored any further.

Sterilants

The sterilants Citrex® and Sporekill® proved extremely effective in reducing growth at the standard rate currently applied in the nursery. Citrex® is the least toxic of all the products tested. This product is recommended in animal feed and drinking water to control internal pathogens (Citrex, 2000). Compared with the control the copper oxychloride and Prasin® treatments proved very effective in controlling growth at the rate of 0.1%, although they were only as effective as the Citrex® and Sporekill® if their dose was doubled **(Table 3)**. The low toxicity levels of Prasin®, however, will favour the continued evaluation of this product in future tests. Products containing copper are not advised due to their persistence as a heavy metal in soils and the toxic effects on fish and aquatic organisms. Bulab® 6044 was the least effective of all the sterilants tested and was only found to be comparably effective at a 2% rate of application. At this rate, however, the product was noted to exhibit an extremely strong smell and had a severe bleaching effect on the PDA medium rendering it completely colourless.

It has been reported that sterilants such as Prasin® and Sporekill®, are more effective in controlling spore germination than on reducing colony growth (Nel and Viljoen, FABI, pers. comm., 2004). In trials conducted at the Forest and Agricultural Biological Institute (FABI), based at the University of Pretoria, these two products showed apparent excellent control in reducing spore germination rates of both *F. circinatum* and *F. subglutinans* f.

sp. *cubensi* (Panama wilt) in liquid suspension media (Nel and Viljoen, FABI, pers. comm., 2004). The value of these products in controlling *F. circinatum* may, therefore, not have been accurately determined using the techniques applied in this trial and tests on these products should be repeated using the techniques adapted by FABI.

Table 3: The interaction between product and application rate on colony growth of the two *Fusarium circinatum* strains tested.

Product	Colony growth (mm) at various application rates		
	Half	Standard	Double
Octave®	0	0	0
Benomyl®	0.36	0	0
Folicur®	0.64	0	0
Tilt®	1.45	0.97	0
Citrex®	9.95	5.29	0.83
Sporekill®	12.58	8.74	3.01
Copper®	18.21	15.81	6.96
Prasin®	29.24	22.35	13.04
Bulab®	51.98	32.91	7.39
Previcure®	43.16	35.93	30.98
Control	41.45		
$LSD_{0.05}$	4.010		

CONCLUSIONS

The two laboratory studies indicate that suitable alternatives to the prohibited fungicide Benomyl may exist for further study *in vivo*. The biological agent, *T. harzianum* (strain Kd), was highly antagonistic towards *F. circinatum*. The fungicides displaying the same level of control as Benomyl were Octave®, Folicur® and Tilt®. Of these three products,

Folicur® and Octave® are less toxic and should be regarded for further tests. The sterilants, Citrix®, Sporekill® and Prasin® ranged from excellent to good control and, although not as effective as Benomyl *in vitro*, can definitely be recommended for future testing *in vivo* due to their low residual toxicity levels in the environment. This is especially true for Citrex®.

Future trials should be designed to test the level of toxicity of these products on young plants in the nursery and for their effectiveness in controlling *F. circinatum* in a soil environment. This study was only able to compare the direct relationship between the pathogen and a controlling agent. The nursery or field situation is more complex because the controlling agent may interact with the host plant or environment with different results.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the following persons for their valuable contributions;

1. Prof. Mark Laing, of the University of KwaZulu Natal, for developing the *Trichoderma harzianum* strain (Kd), and Dr. Mike Morris for making the strain available (as Eco-T®) for testing,
2. Mr. Jacques Szyndralewicz for sponsoring Prasin®, and Mr. Cobus Erasmus for sponsoring Citrex® tested in these trials.
3. Izette Greyling, Lorenzo Lombard and Bernice Porter, of the University of Pretoria, for their assistance with the media preparation and growing up the *F. circinatum* isolates used.
4. Vijay Bandu and Belinda White, at the University of KwaZulu Natal, for their assistance with the electron microscopy work carried out.
5. Management staff at the Sappi Ngodwana nursery for making several of the chemicals in their store available for testing, and for the information supplied on the rates applied.

Chapter III

Shoot morphology and site climate affect re-establishment success of *Pinus patula* in South Africa

Mitchell, R. G., Zwolinski, J. and Jones, N. B., 2005. Shoot morphology and site climate affect re-establishment success of Pinus patula in South Africa. Southern African Forestry Journal 205: 13 – 20.

ABSTRACT

Operational experience has indicated that *Pinus patula* generally survives poorly on sub-optimal growing sites in South Africa, particularly when planted as cuttings. This observation will become increasingly important as softwood species of genetically selected stock are deployed as cuttings in preference to seedlings of lower genetic value. Methods to improve survival of this species are yet to be fully defined. Production of *P. patula* seedlings is currently based on broad prescriptions, which commonly include raising containerised stock for 5 to 7 months in the nursery and limiting planting to the early and late summer months. Little research has been conducted on determining optimal plant dimensions at planting or defining sites that are of high or low planting risk. This paper describes some effects of site climate and shoot morphology on post-planting survival and is the first to provide suggested guideline dimensions for *P. patula* cutting and seedling nursery stock raised in containers, 80 – 90 ml in volume. Further work is required to establish the validity of these dimensions.

INTRODUCTION

Creating conditions that will be conducive to maximum survival of new plantings is essential in optimizing site potential. This becomes increasingly important when improvements are made to the genetic potential of the material planted. Poor survival of improved material will negate the breeding and production efforts inputted prior to planting to improve the yield from the site.

The most important softwood species planted in South Africa is *Pinus patula* (Bester *et. al.*, 2000). Local research efforts on the factors affecting the re-establishment success of this species indicates that post-planting temperature (Morris, 1991), disturbance to the root system before planting (Morris, 1993), and pests and diseases (Atkinson, 1997; Allan *et. al.* 2000; Crous, 2004) contribute significantly towards a reduction in field survival. It has been acknowledged, however, that little is known about defining quality of containerised plants in South Africa especially with cuttings (Zwolinski and Bayley, 2001). The limited research has been conducted on seedling quality and this suggests that younger aged seedlings survive better than older, root-bound plants (Morris, 1994). From this and other work, it has been proposed that *P. patula* seedlings should be grown for 5 to 7 months if raised in containers 80 ml in volume (Zwolinski and Bayley, 2001). Such prescriptions, however, do not include the optimal size for optimal aged seedlings or the effect of plant size under different site conditions.

During the 2002/3 planting season, four trials were conducted to test the effect of fungicide application at planting on survival rate. The results, however, indicated that fungicide application had no influence on field survival (Mitchell and Jones, 2005) suggesting that factors, other than disease, were influencing survival in these trials. Using nursery measurements obtained prior to planting and climate data obtained from the field during the first year after establishment for each of the four trials, the authors aimed to investigate the effect of site climate and shoot morphology at planting on the re-establishment success of these trials.

MATERIALS and METHODS

The four trials used in this study were planted at two sites that differed in expected rainfall and temperature. The two sites were planted one month apart at the end of the softwood planting season, which generally extends from October to April. At each site cuttings and seedlings were planted in separate trials adjacent to each other. Each trial consisted of 3000 plants. The details of the trial sites are shown in **Table 1.**

Table 1. Trial sites and planting details

Plantation name	Helvetia	Driekop
Location (Grid reference)	25° 33' 10" S 30° 19' 25" E	24° 54' 48" S 30° 49' 10" E
Nearest town	Waterval Boven	Graskop
Planting date	06/03/2003 (cuttings) 08/03/2003 (seedlings)	08/04/2003 (cuttings) 10/04/2003 (seedlings)
Elevation	1700 m	1440 m
Mean annual temperature	14.8 °C	15.9 °C
Mean annual precipitation	775 mm	1233 mm
Previous species	*Pinus patula*	*Pinus patula*
Slash management	No burning, slash distributed	No burning, slash distributed
Weed control	Pre-plant chemical spray	Pre-plant chemical spray
Planting density	3 m x 2 m (1667 stems/ha)	3 m x 2 m (1667 stems/ha)

Nursery procedure

The cutting material used in the trial series was obtained from the Komatiland Forests nursery, near Sabie. The material was transferred to the Sappi Forests nursery, at Ngodwana, soon after rooting where it was raised adjacent to seedling material obtained from the Ngodwana nursery. The cutting material comprised two open pollinated families selected for improved growth. The plants in the nursery received the same treatment

consisting of daily watering and two applications of soluble N:P:K fertiliser a week, at a rate of 1%. Fertiliser applications alternated between Hortichem Orange® (2:3:2 (42)) and Hortichem Blue® (5:2:4 (43)). Plants were raised in containers that were used operationally for each plant type. Cuttings were produced in the Unigro 98 tray consisting of square-shaped cavities, 90 ml in volume. The seedlings were raised in the Sappi 49 tray with round-shaped cavities, 80 ml in volume. Both containers included root trainers (raised internal ridges) to prevent root spiraling and did not possess root-pruning side-slits. The cuttings were raised for a period of 8 and 9 months, and the seedlings for a period of 6 and 7 months, before planting. These periods were considered optimal for each plant type.

Prior to planting, all plants were assessed for nursery root collar diameter (RCD) to the nearest 0.01 mm and height (Ht) to the nearest 1 cm. From these two measurements, a sturdiness ratio (SR) was derived by dividing Ht (mm) by RCD (mm). When the seedlings were two–months-old, the number of cotyledons (first set of needles to appear after germination) per seedling was recorded. A record of the individual plant characteristics assessed was maintained for each plant planted in the trial as well as its position in the field.

Field monitoring

Site climate was monitored hourly by recording temperature and relative humidity for the first 12 months after planting using a Hobo® logger (Onset Computer Corporation)

positioned at chest height (approximately 1.3 m) above ground level at each site. The logger was housed in an especially designed well ventilated pole, and was reported to be accurate to within 0.5 °C of the temperature recorded in a standard Stevenson screen (S. Dovey, ICFR, pers. comm., 2005). Rainfall was recorded daily at the plantation office located close to each trial site. All trials were assessed for survival monthly. Plants were individually assessed and recorded as being either dead or alive. The final survival assessment was carried out at month 12.

Data analysis

Each of the four trials consisted of 120 plots planted to 25 trees. Survival was calculated per plot as a percentage. In order to assess the overall effect of plant type, site and the interaction between the two on survival, the four trials were analysed together, using analysis of variance (ANOVA). All survival percentages were transformed using angular transformation, to normalize the data, prior to analysis using GenStat for Windows 5th Edition.

Assessing the effects of shoot morphology on survival was carried out separately for each trial. The 3000 plants within each trial were grouped according to RCD classes of 0.1 mm, Ht classes of 1 cm, and SR classes of 5 mm. Cotyledon number was grouped according to the number of needles counted (i.e. 4, 5, 6, 7 and 8). In each case, the plants that were alive in month 12 were expressed as a percentage of those planted. To avoid large deviances in survival due to an insufficient sample size, classes represented by

fewer than 10 plants were grouped within the next class. Simple linear and quadratic regression analyses were then performed on all survival data with RCD, Ht, SR and cotyledon number separately in order to determine the type of relationship that each may have had on survival.

RESULTS

Site climate and plant type

Analysis of the transformed data from all four trials indicated that survival differences between sites were highly significant ($p<0.001$) by the end of 12 months since planting. Differences in plant type (cuttings and seedlings) survival was non-significant ($p=0.245$) although there was a significant plant type by site interaction ($p<0.001$), with the cuttings performing slightly poorer on the Helvetia site and slightly better on the Driekop site **(Figure 1)**.

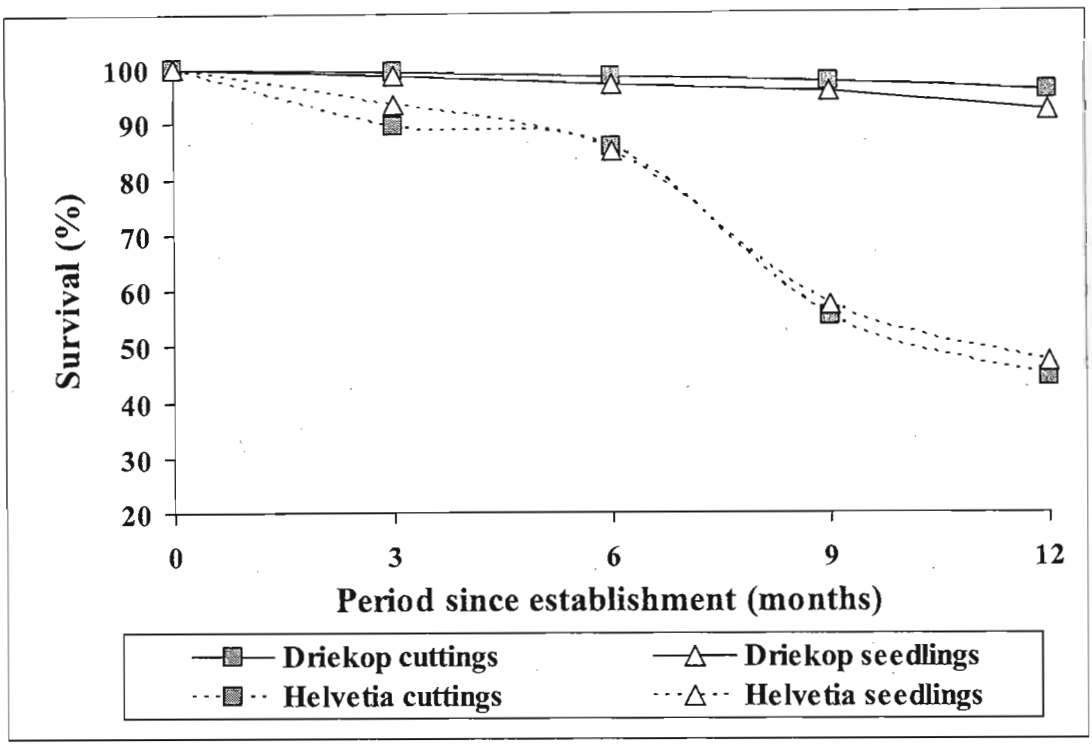


Figure 1. Quarterly plant survival assessed for both cuttings and seedlings on the two planting sites.

The monthly mean maximum temperatures recorded at the two sites were similar for the first year after planting **(Figure 2)**. Furthermore, extreme high temperatures determined by the number of hours greater than 25 °C recorded in the first year after planting, were also similar between Driekop (821 hours) and Helvetia (855 hours). Mean minimum temperature, however, was lower at Helvetia (7.05 °C) compared with Driekop (9.39 °C) and was also characterised by greater extremes during the winter months (May – August) **(Figure 2)**. The number of hours that the temperature dropped below 5 °C in the first year after planting at Helvetia was 1031 compared with 454 at Driekop.

Mean relative humidity was approximately 10% less at Helvetia **(Figure 3)** and, similar to minimum temperature, was characterised by greater extremes. Humidity fell below 30% for 1781 hours at Helvetia, and for 661 hours at Driekop, during the first year after establishment.

In the 5-6 months between the commencement of the planting season in October 2002 and planting at each site, 643 mm of rain had fallen at Helvetia and 554 mm had fallen at Driekop **(Table 2)**. Rainfall for the first 12 months following planting, however, was 684 mm at Helvetia compared with 1346 mm at Driekop. Moreover, the Driekop site continued to receive rainfall during the winter months (May, June, July and August), whereas Helvetia received either no or very little rain during these months of the year.

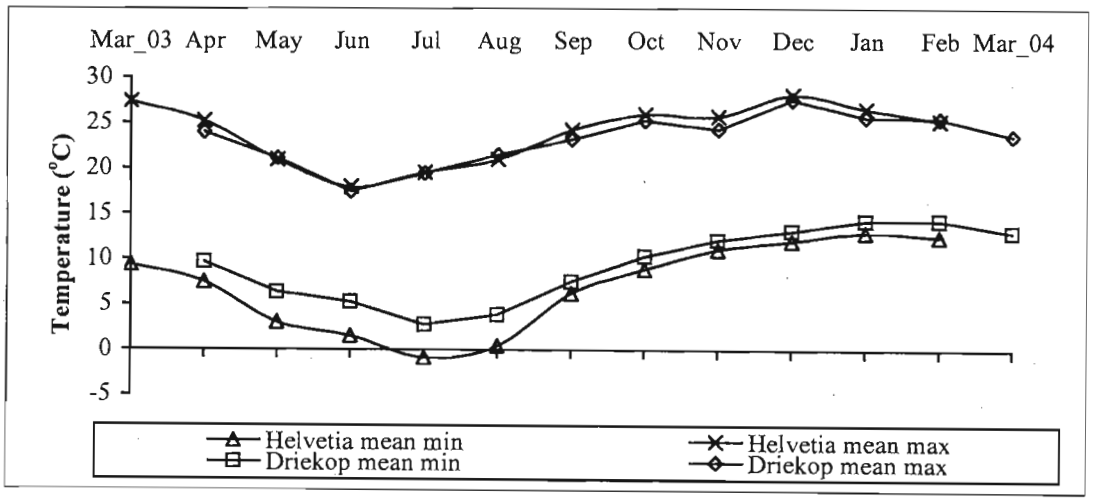


Figure 2. Mean monthly minimum and maximum temperatures for both sites for the first 12 months after planting.

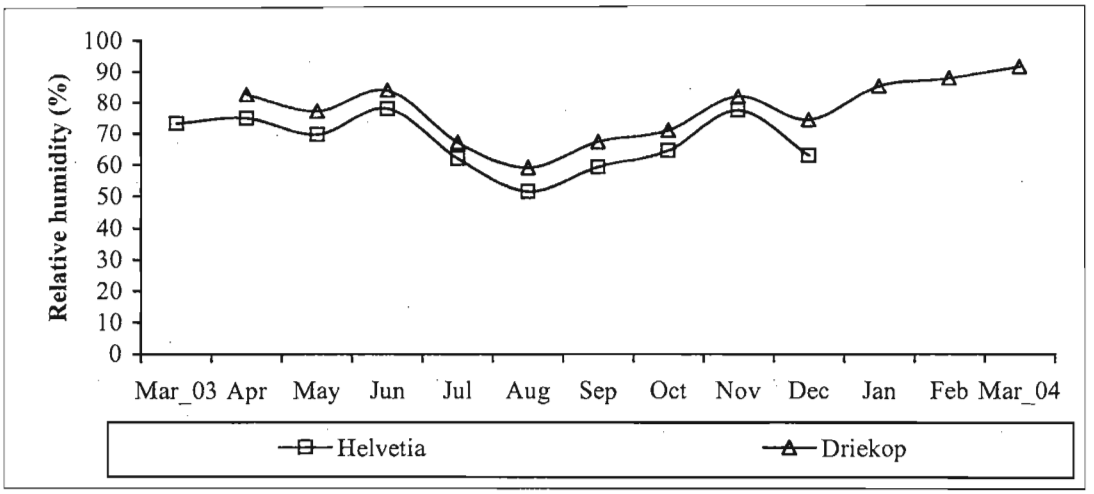


Figure 3. Mean monthly humidity for both sites over the first 12 months.

Table 2. Monthly rainfall measured at both sites prior and post planting.

Month	Helvetia	Driekop
October '02	94	119
November '02	74	44
December '02	178	93
January '03	153	109
February '03	144	127
March '03	36	181
April '03	8	17
May '03	1	23
June '03	6	65
July '03	0	14
August '03	6	8
September '03	6	55
October '03	45	70
November '03	101	94
December '03	158	124
January '04	161	218
February '04	157	304
March '04	79	355

Shoot morphology

The results of the regression analysis of survival as a function of RCD, Height and SR showed that these characteristics were significantly associated with survival (quadratic relationship) on the site where sub-optimal survival was obtained (Helvetia) but were non-significant at the Driekop site, where tree survival was significanty better **(Table 3)**. The effect of cotyledon number on survival was not significant at both sites.

Table 3. The results of the best fit (quadratic at Helvetia and linear at Driekop) regression analyses of 12-month survival with shoot morphology.

Site	Criteria measured	Seedlings		Cuttings	
		F prob	R^2	F prob	R^2
Helvetia (sub-optimal conditions)	Root collar diameter	<0.001	0.73	<0.001	0.80
	Height	<0.001	0.83	<0.022	0.24
	Sturdiness ratio	<0.001	0.78	0.001	0.58
	Cotyledon number	0.433	0.00	NA	NA
Driekop (optimal conditions)	Root collar diameter	0.412	0.00	0.141	0.06
	Height	0.338	0.00	0.156	0.06
	Sturdiness ratio	0.479	0.00	0.105	0.11
	Cotyledon number	0.458	0.00	NA	NA

NA = Not applicable

Root collar diameter at Helvetia

The survival relative to RCD of cuttings at Helvetia improved in a curvi-linear fashion up to a diameter class of 3.2 mm, thereafter, the variation in survival around the fitted model increased **(Figure 4)**. Seedling RCD classes on the other hand, displayed a parabola-shaped curve indicating when plants were either undersized or oversized **(Figure 5)**. On this site, the best survival range was achieved for RCD between 2.8 and 3.2 mm for cuttings, and 1.8 mm and 2.2 mm for the seedlings.

Stem height at Helvetia

The relationship of cutting height on survival was not as significant as that of RCD **(Figure 6)**. It was difficult to define optimal height for cuttings at the Helvetia site but it would appear that cuttings above 7 cm in height survived better. The highly significant quadratic relationship between stem height at planting and survival for the seedling data **(Figure 7)** indicated that height may be very important in defining seedling quality. The optimal range extended from 10 to 15 cm in this case.

Sturdiness ratio at Helvetia

The strong relationship that existed between sturdiness ratio and survival indicates that increased sturdiness ratios, i.e. taller plants relative to stem diameter, had a significant negative effect on cutting survival **(Figure 8)**. Similar to the effect of RCD and height on

seedling survival, the relationship between SR and seedling survival at Helvetia was parabolic **(Figure 9)**. It would appear that SR for cuttings should not exceed more than 5 cm in height for every 1 mm in stem diameter (i.e. 50) and that seedling heights should be between 5 and 8 cm for every 1 mm in stem diameter (i.e. 50 – 80).

Cotyledon number at Helvetia

Although there was no relationship with cotyledon number and survival, there was a significant relationship between cotyledon number and seedling size **(Table 4)**. Using the data at Helvetia, the relationship with seedling height was weaker and could be described as linear ($p=0.052$, $R^2=0.10$), while the relationship with RCD was stronger and could be described as quadratic ($p<0.001$, $R^2=0.40$).

Table 4. Mean seedling dimension at planting per cotyledon-number category at Helvetia.

Cotyledon number	RCD (mm)	Height (cm)
4	1.58	10.04
5	1.63	10.22
6	1.65	10.52
7	1.75	10.84
8	1.79	11.82

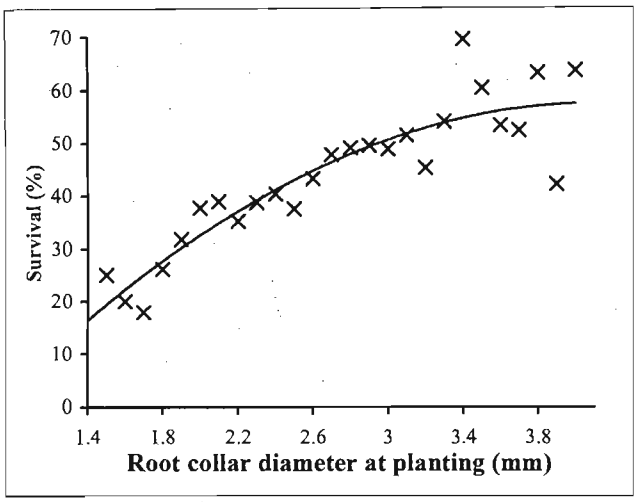


Figure 4. Relationship between cutting root collar diameter at planting and 12-month survival.

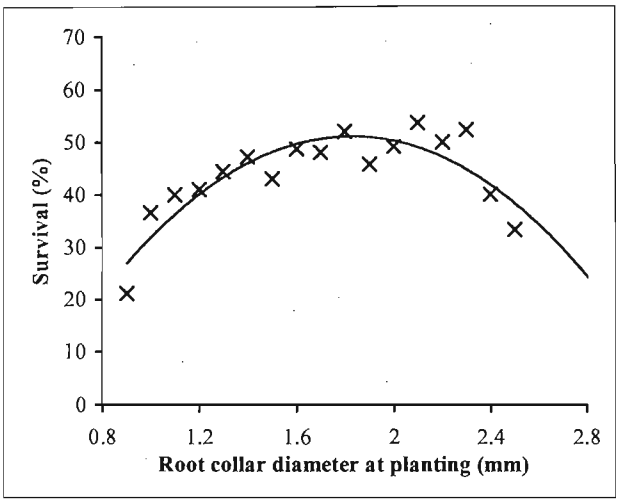


Figure 5. Relationship between seedling root collar diameter at planting and 12-month survival.

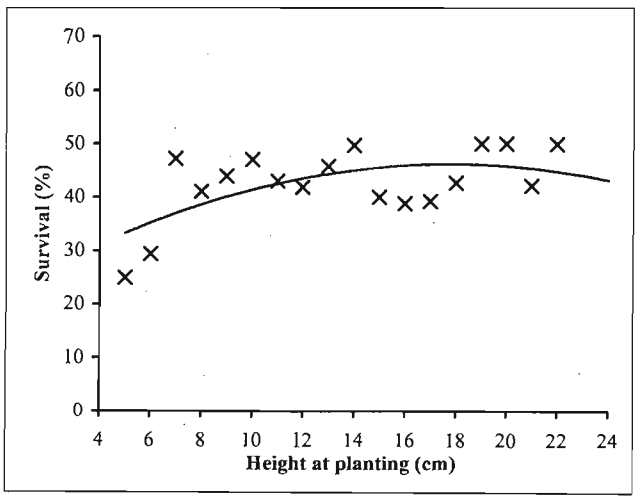


Figure 6. Relationship between cutting height at planting and 12-month survival.

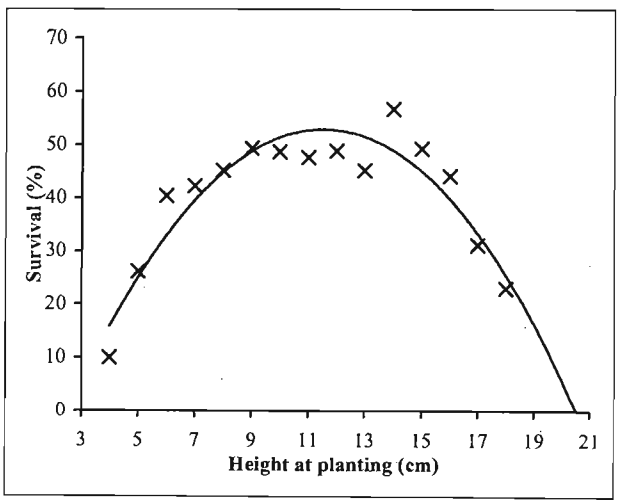


Figure 7. Relationship between seedling height at planting and 12-month survival.

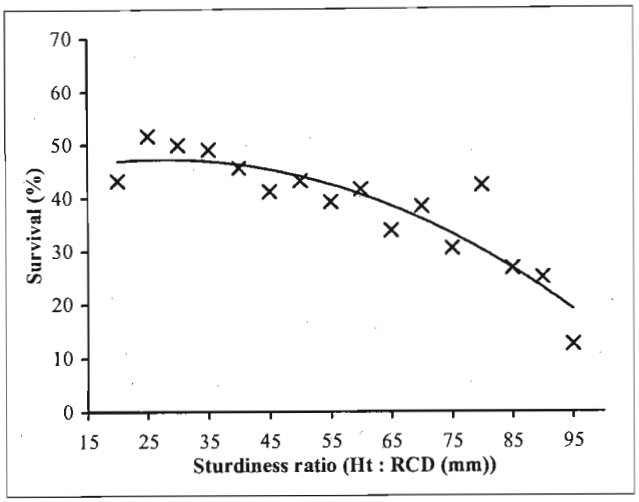


Figure 8. Relationship between cutting sturdiness ratio at planting and 12-month survival.

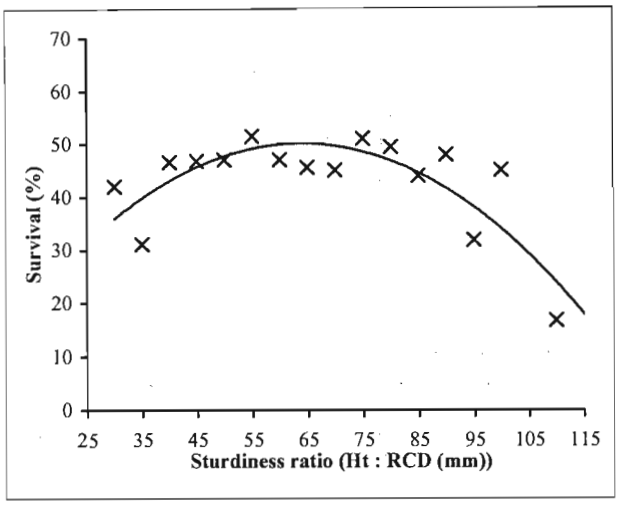


Figure 9. Relationship between seedling sturdiness ratio at planting and 12-month survival.

DISCUSSION

Differences in survival between the two sites appear to be driven by differences in climate. Temperature extremes are known to have a greater influence on post-planting mortality than averages (Morris, 1991; McTague and Tinus, 1996; Allan and Higgs, 2000). The lower air temperatures observed at Helvetia, particularly when below 3 °C, favour the formation of frost (Pallett, 1999). The probability that the plants would have been subjected to increased severity and longer periods of frost (freeze) damage would, therefore, have been greater at Helvetia. Low atmospheric humidity, particularly when less than 30% and for extended periods, has also been shown to influence survival (McTague and Tinus, 1996). The data from the present study may, therefore, support the use of relative humidity to define planting risk. Consequently, the extremes in low

temperature, insufficient rainfall during the winter months, and frequent periods of low atmospheric humidity would suggest that Helvetia was sub-optimal, in comparison to Driekop, for late season re-establishment.

The fact that plant morphological descriptors had no influence on the survival at the Driekop site, but influenced survival significantly at the Helvetia site indicates that the less extreme climate at Driekop masked the effect that shoot morphology may have had on survival at this site. This highlights the importance of understanding site types and of planting correctly graded stock especially on marginal sites in order to maximize survival. This also indicates that the easily measurable morphological parameters (Ht and RCD) can probably be used to define containerised nursery stock quality of *P. patula* operationally.

Root collar diameter is frequently cited as a good measure to use in assessing survival due to the close positive relationship with root mass (Thompson, 1985; Mexal and Landis, 1990; Rose *et al.*, 1990; 1991; Ritchie *et al.*, 1993; Zwolinski *et al.*, 1994; McTague and Tinus, 1996). From the current study, it would seem that the cuttings planted at Helvetia support this statement (RCD R^2=0.80, height R^2=0.24). Sturdiness ratio correlated significantly with survival for both plant types (cuttings R^2=0.58 and seedlings R^2=0.78) indicating that the manner in which RCD and height relate to each other is also important to survival. This implies that plant height should not be excluded from assessing cutting quality. Elsewhere, the relationship between sturdiness ratio and survival of *P. patula* seedlings has been shown to be very similar (R^2=0.769) (Zwolinski

and Bayley, 2001) to that measured in the Helvetia study. Maximum sturdiness ratios generated at Helvetia (1 mm RCD : 5 cm height (cuttings); 1 mm RCD : 5 - 8 cm height (seedlings)) are also very similar to the maximum ratio suggested for containerised *Picea mariana* (Black spruce) seedlings (1 mm RCD : 6 cm height) (Thomson, 1985).

Very large RCD has been shown to reduce survival of container grown seedlings due to root binding, which appears to be related to a reduction in the potential for new root growth after planting (South *et al.*, 2005; South and Mitchell, 2005). The seedlings, with root collar diameters greater than 2.2 mm in the Helvetia study may therefore, have been root bound at the time of planting. The direct relationship that seedling cotyledon number had on RCD, suggests that, although this variable did not relate directly with survival, there may be an indirect relationship worth exploring in future studies.

It has been reported that seedling height is an important morphological attribute when planting on dry sites with an increase in height resulting in a decline in survival (Thompson, 1985; McTague and Tinus, 1996). Thus, it was not surprising that seedling height at Helvetia appeared to be very important (R^2=0.83) with seedlings taller than 15 cm showing a decline in survival. Taller plants, which are likely to have larger leaf areas, generally transpire at greater rates (Rose *et al.*, 1990) suggesting that the taller seedlings in this trial would most likely have been under increased stress due to the lack of available water. It is for this reason that shorter, stockier plants, with smaller leaf areas, have been prescribed on more arid sites (Mexal and Landis, 1990).

It was encouraging to observe that cutting and seedling survival was similar providing further evidence (Mitchell *et. al.*, 2005) that *P. patula* cutting survival should not hinder commercial deployment. Although the significant plant type by site interaction may indicate that cuttings are more prone to mortality under sub-optimal conditions, the difference in 12-month survival between the two plant types at Helvetia were very small (cuttings 43.93%, seedlings 46.97%).

Operational considerations

Currently, due to insufficient knowledge on optimal plant dimensions for different sites, it would seem that all nursery stock should be raised to dimensions specified to achieve optimal survival under sub-optimal planting conditions. Once optimal plant dimensions have been defined for different site conditions, plants can be raised to suit the specified site. These studies indicate specifications useful in achieving optimal survival under harsh climatic conditions, and provide a starting point for further studies to determine their validity for operational use.

In addition to planting the correctly graded nursery stock on sub-optimal sites, it may be necessary to define the optimal "planting window" (planting period) for different site types to improve survival (Landis, 2002). Due to the higher planting risk at sites such as Helvetia, the "planting window" may be narrower, and different, compared to sites such as Driekop. The trials in this study were established towards the end of the softwood planting season. In the case of Helvetia, the negative effects of low temperature and little

or no rain following planting, may have been avoided had the study site been established earlier in the planting season. The implications on the planning and timing of site preparation and planting activities will be directly influenced by the optimal "planting window".

CONCLUSIONS

The data obtained from recording site climate and plant morphology at planting in the 4 studies, yielded substantial information about these effects on post-planting survival success. It would seem that the extremes in low temperature and humidity, as well as lack of rainfall can result in severe mortality. In order to improve survival after out-planting it is proposed that optimal planting periods be defined for different sites. Furthermore, it was observed that planting correctly graded planting stock is essential when maximizing post-planting survival under sub-optimal planting conditions. Planting should, therefore, take place during the optimal planting periods using nursery stock designed to survive well under most climatic conditions.

Survival was best achieved on the sub-optimal site for cuttings grown in cavities 90 ml in volume, if root collar diameter was between 2.8 mm – 3.2 mm and stem height was greater than 7 cm at planting. Seedlings, grown in cavities 80 ml in volume, survived best if the root collar diameter was between 1.8 – 2.2 mm with a stem height of 10 – 15 cm at planting. The manner in which RCD and Ht related (SR) also proved important. Cutting height should not be greater than 5 cm for ever 1 mm RCD and seedling height should be between 5 and 8 cm for every 1 mm RCD. These criteria provide nurserymen with

guidelines as to what *P. patula* seedling and cutting dimensions might be at planting. Further studies are required to verify these guidelines. Cuttings and seedlings survived equally well indicating that comparable survival can be expected operationally if good quality plants are raised, handled and planted correctly.

ACKNOWLEDGEMENTS

The authors acknowledge Ms. L. McNamara, of Global Forests Products, for arranging and assisting with the planting of the Driekop trials and Mr. C. Wentzel, of Komatiland Forests, for providing the rooted cutting material. Field assistants, T. Zulu, G. Malapane, R. Thibela and J. Phonela are acknowledged for carrying out the routine assessments at both sites.

Chapter IV

Root volume and raising period affect field performance of *Pinus patula* cuttings in South Africa

Mitchell, R. G., Zwolinski, J., Jones, N. B. and Bayley, A. D., 2005. Root volume and raising period affect field performance of Pinus patula cuttings in South Africa. Southern African Forestry Journal 204: 15 – 21.

ABSTRACT

The propagation of pines through cuttings has become a commercial means of rapidly multiplying improved genetic material for operational use in forestry companies. Cuttings of pines are produced entirely in containers in South Africa. Containers, however, can negatively affect plant growth and post-planting field performance if plants are allowed to grow beyond the constraints of the root cavity. The aim of this study was to determine the effects of tray type and plant age on the field performance of *Pinus patula* rooted cuttings. Field assessments indicate that the combination of greater root mass at planting and increased media volumes improved field growth with the most significant response observed in stem diameter. Factors responsible for producing greater root dry mass at planting were increased media volume and a longer raising period in the nursery. Seven years after planting, cuttings with the largest root mass at planting (0.560 g) were 27% larger in individual tree volume than trees produced from cuttings having the smallest root mass at planting (0.159 g). Field survival was exceptionally good and did not differ among nursery treatments.

INTRODUCTION

Raising planting stock in containers (trays) is now widely accepted as an efficient and practical method of producing plants for field establishment. Under variable planting conditions in South Africa, containerised planting stock may yield better post-planting survival and early growth than open-rooted stock. Furthermore, when one seed is sown per cavity, seed use efficiency is improved and small containerised plants take less time to produce compared with large open-rooted plants (Barnett and Brissette, 1986). However, problems such as root deformities in over-sized plants have often been cited as the disadvantages of the containerised system (Grene, 1978; Tinus, 1978 a, b; Bayley, 1998).

Even when plants are dispatched timeously, the shape and volume of the cavity appears to have an effect on root form and development. Previous studies testing the effect of various container types on the performance of *Eucalyptus grandis* seedlings, indicate that leaf surface area, root dry mass, root : shoot ratio, as well as height and growth rates after transplanting are all affected by tray type (McCubbin and Smith, 1991). In other studies the ability for newly planted seedlings to rapidly generate roots impacted positively on field survival (Bayley, 1995, 1998; Morris, 1990), and subsequent growth (Tinus, 1978 b). If factors such as tray type and root architecture influence seedling performance, the effect with cuttings may be even more important given the more fragile nature of their root systems.

The aims of this study were to assess the field survival and growth of *P. patula* cuttings raised for different lengths of time in several types of containers. Containers were selected based on their shape and volume.

MATERIALS and METHODS

Plant materials

In May 1994 *Pinus patula* seedling hedges were established using a Zimbabwe Forestry Commission (ZFC) seedlot (P16347) comprising of a mix of open pollinated families. Family representation in the hedge bank was randomly distributed. When the hedges were approximately 15-months-old from their date of sowing, shoot cuttings were harvested for the trial. Shoots were set in July and September 1995 to achieve cuttings of two different ages at planting. Two *P. patula* seedling controls (age 6 and 7 months) were obtained from production blocks at the Ngodwana Nursery.

Tray types

There were five types of containers, each differing in cavity volume (**Table 1**). All trays used for raising the cuttings were treated with a copper root-pruning agent, Spin Out® (active ingredient, cupric hydroxide, 116 gl^{-1} latex carrier; Griffin Corporation) before filling with media. Thus all the cutting treatments had a pruned root system. In addition to chemical root pruning, the BCC© trays have large slits in the sides of the inserts to air-prune roots and a large opening at the base of each cavity. The cuttings set in the various tray treatments were raised for 7 and 9 months before planting. The seedling controls were raised in the Sappi standard tray and were only air-pruned at the bottom of the plug. While the containers used in this trial differed in colour, cavity shape, cavity volume and type of pruning application provided only container-type and age differences were considered as the experimental treatments **(Table 1)**.

Table 1. Specifications of the containers used to raise the cuttings.

Container types[1]	Colour	Cavity shape	Cavity diameter (mm)	Cavity length (mm)	Root volume (ml)[2]	Plant density (#/m^2)	Type of root pruning applied[3]
BCC SideSlit 81©	Black	Square	39	85	60	546	Air + Chemical
Sappi 49 (standard)	White	Round	39	80	80	423	Chemical
Unigro 98©	Black	Square	37	100	90	450	Chemical
Unigro 72©	Black	Square	52	100	125	330	Chemical
Sappi 49 (deep)	White	Round	39	130	130	423	Chemical

[1] All containers were used for raising the cuttings, only the Sappi standard tray was used for the seedlings

[2] The BCC plug, although 100 ml in volume, was recorded as 60 ml due to excessive media loss through the large side slits and opening at the bottom of each cavity

[3] Lateral root pruning was only applied to the cuttings and not to the seedlings

Nursery procedures

Growing media consisted of 12 mm-sieved, composted pine bark. Cuttings were set without the use of rooting hormone. Once set, cuttings were placed in a fibreglass greenhouse where the shoots received intermittent mist. Temperatures in the rooting medium were maintained above 25 °C. Cuttings remained in the rooting environment for a period of 90 days before being moved out into the nursery tunnels where they received normal nursery treatment consisting of daily irrigation. In addition, two applications per week of soluble N:P:K fertiliser were applied at a rate of 1%. Fertiliser applications alternated between Hortichem Orange® (2:3:2 (42)) and Hortichem Blue® (5:2:4 (43)). The plants were raised at the Ngodwana Nursery.

Nursery measurements

Plant dimensions were determined immediately prior to planting the trial by selecting nine plants down the centre two rows of a randomly selected tray in each treatment and

measuring root collar diameter (RCD) and plant height. Root dry mass (RDM) at the time of planting was determined by assessing a further ten randomly selected plants per treatment, from another tray. To obtain RDM, root plugs were washed of all excess bark medium and roots were removed from the base of the cutting using a sharp blade. Once removed, the roots were oven-dried for 48 hours at 70 °C, before being weighed.

Field design and planting

The trial was planted in April 1996 on the Sappi Elandshoogte plantation. The experimental design was a Randomised Complete Block with 5 replications **(Table 2)**. The cutting treatments consisted of plants of two ages, raised in 5 different tray types. Two seedling controls were included, also of two ages, but were raised in one container type. Individual plots consisted of 16 trees (4 x 4 tree plots). The distance between trees at planting was 2.4 m. Plants were planted using the "puddle" planting technique where one to two litres of water is added to the planting pit at the time of planting creating a mud slurry, into which the plants are planted. The experimental area had been burnt one month before planting. Climatic details of the site are summarised in **Table 2.**

Table 2. Trial design and land type details

Trial design	Randomised Complete Block
Number of treatments	12
Number of replications	5
Plot size	4 x 4 tree plot
Tree spacing	2.4 m x 2.4 m
Elevation (m.a.s.l.)	1800 m
Mean annual temperature	13.8 °C
Mean annual precipitation	1140 mm

Field assessments

Individual tree heights were measured for the first time at 2.5 years after planting. The trial was assessed again at 5 years and 7 years after planting. During the second and third assessment, diameter at breast height (DBH) and height was measured. From the DBH and height data collected, individual tree volume was calculated using the Schumacher and Hall model (Bredenkamp, 2000).

Statistical analysis

Survival percentages were arcsine transformed prior to analysis (Zar, 1984). An analysis of variance (ANOVA), using the Genstat 5, version 3.2® Statistical Programme (Genstat, 1995) was performed on all data. The least significant difference (LSD) values were calculated at the 5% level of significance. All cutting data were contrasted with all seedling data to compare cutting with seedling performance. Within cuttings, the effect of tray type and raising period was assessed with contrasts. Simple linear regression analyses were performed to determine the relationship among various plant attributes at planting as well as between the plant attributes at planting (explanatory variables) and the field-measured criteria (dependant variables).

RESULTS and DISCUSSION

Nursery performance

At the time of planting the older plants tended to be larger with a larger root dry mass, irrespective of the tray type (**Table 3**). Simple linear regression analysis on the nursery data indicated that root dry mass (RDM) at planting was significantly ($p<0.001$) influenced by both plant age ($R^2 = 0.50$) and media volume of 9-month-old cuttings ($R^2 = 0.11$). The likely reason that no relationship existed between root dry mass and the amount of media in the younger cuttings is that the plugs were not fully colonised by 7 months. Younger cuttings had therefore not been able to take full advantage of the increased root growth area offered by the increase in media.

Table 3. Treatment means resulting from an ANOVA of various nursery parameters measured prior to planting

Treatments	Height at planting (mm)	Root collar diameter at planting (mm)	Root dry mass at planting (g)
Cuttings - Unigro 98 - 7 months	103.89 (10.9)	2.94 (0.175)	0.235 (0.048)
Cuttings - Unigro 98 - 9 months	216.22 (16.8)	3.75 (0.169)	0.459 (0.054)
Cuttings - Unigro 72 - 7 months	110.22 (8.4)	3.07 (0.112)	0.314 (0.053)
Cuttings - Unigro 72 - 9 months	275.67 (27.9)	3.96 (0.102)	0.560 (0.076)
Cuttings - Sappi std - 7 months	81.33 (7.64)	2.84 (0.175)	0.159 (0.012)
Cuttings - Sappi std - 9 months	244.78 (37.0)	3.79 (0.462)	0.459 (0.059)
Cuttings - Sappi deep - 7 months	95.56 (5.66)	3.22 (0.154)	0.182 (0.025)
Cuttings - Sappi deep - 9 months	308.56 (25.1)	4.77 (0.560)	0.414 (0.039)
Cuttings - BCC - 7 months	131.89 (6.1)	2.98 (0.082)	No data available
Cuttings - BCC - 9 months	203.56 (23.0)	3.52 (0.207)	0.232 (0.068)
Seedling control - 6 months	223.89 (11.0)	2.93 (0.083)	0.298 (0.026)
Seedling control - 7 months	212.78 (13.2)	2.80 (0071)	0.388 (0.019)

Values in parentheses represent the standard error of the mean

Field performance

Survival

Overall survival was above 95% in month three and therefore the trial was not blanked. The outstanding field survival may have been due to high soil moisture noted at the time of planting and mean annual precipitation in excess of 1000 mm per year at the chosen site. At 7 years of age survival was still high and differences between treatments were not statistically significant **(Tables 4 and 5)**. The similar survival between the cutting and seedling treatments has been observed in other trials (Mitchell *et al.*, 2004b).

Growth

Treatments differed significantly in height, diameter and volume at 7 years **(Table 4)**. The mean of all cutting treatments was significantly larger for stem diameter ($p=0.043$) and ranked higher for height and volume compared with the seedlings **(Table 5 and Figures 1 - 3)**. Although the stock number of the seedling material used in the trial was not recorded at the time of planting, all seedling material being deployed from the Ngodwana Nursery in the month of planting (from which the control was taken) was genetically superior to the cutting material (A. Kanzler, Sappi Forests Research, pers. comm., 2005). The observed differences in field diameter performance between the two plant types were therefore likely due to reasons other than genetic differences. For example, seedlings were raised in untreated trays, whereas cuttings were raised in trays treated with a copper root-pruning agent that has been shown to promote field growth (Bayley, 1998). Furthermore, the fact that cuttings generally grew better in diameter than seedlings in this trial was encouraging as it has recently been reported that *P. patula* hedges mature rapidly resulting in a decline in cutting quality (Mitchell *et al.*, 2004a). Although the trial was not assessed for stem form, it was noticed that cuttings produced straighter, more uniform stems, when compared with the seedlings.

Table 4. The effect of container/plant type on survival, height, diameter and stem volume seven years after planting (treatments are sorted in descending order according to tree volume) for treatment combinations.

Treatment	Survival (%)	Height (m)	DBH (cm)	Volume (m^3)
Cuttings - Unigro 72 (125 ml) 7 months	87.5 *(71.8)*	13.314	18.042	0.1283
Cuttings - Unigro 72 (125 ml) 9 months	90.0 *(73.7)*	13.099	17.920	0.1240
Cuttings - Sappi std (80 ml) 9 months	93.7 *(81.1)*	13.087	17.694	0.1209
Cuttings - Sappi deep (130 ml) 9 months	91.2 *(76.6)*	12.640	17.569	0.1162
Cuttings - Unigro 98 (90 ml) 7 months	96.2 *(83.0)*	12.650	17.392	0.1137
Cuttings - BCC (60 ml) 7 months	91.2 *(77.4)*	12.523	16.671	0.1066
Seedling control (80 ml) 6 months	85.0 *(72.3)*	12.555	16.550	0.1062
Seedling control (80 ml) 7 months	88.7 *(72.8)*	12.408	16.760	0.1060
Cuttings - Sappi deep (130 ml) 7 months	92.5 *(75.9)*	12.685	16.638	0.1058
Cuttings - Unigro 98 (90 ml) 9 months	87.5 *(71.8)*	12.186	16.843	0.1018
Cuttings - BCC (60 ml) 9 months	85.0 *(72.8)*	12.364	16.630	0.1012
Cuttings - Sappi std (80 ml) 7 months	88.7 *(74.7)*	12.304	16.504	0.1005
$LSD_{0.05}$	*(13.11)*	0.7939	0.9470	0.01627

Transformed survival percentages shown in italicised parenthesis

Table 5. Analysis of variance of the effects of container type and raising period (plant age) on the growth measurements at seven years after planting.

Variate: Survival (transformed)	d.f.	s.s.	m.s.	v.r.	F. prob.
Replication	4	1594.0	398.5	2.70	0.043
Contrast (Seedlings vs. Cuttings)	1	91.8	91.8	0.62	0.434
Contrast. Container type	4	169.5	42.4	0.29	0.885
Contrast. Plant age	2	24.4	12.2	0.08	0.921
Contrast. Container x age	4	451.6	112.9	0.77	0.553
Residual	44	6488.5	147.5		
Total	59	8819.8			
Variate: Height	d.f.	s.s.	m.s.	v.r.	F. prob.
Replication	4	9.9676	2.4919	6.42	<0.001
Contrast (Seedlings vs. Cuttings)	1	0.3470	0.3470	0.89	0.349
Contrast. Container type	4	4.0221	1.0055	2.59	0.049
Contrast. Plant age	2	0.0590	0.0295	0.08	0.927
Contrast. Container x age	4	2.2488	0.5622	1.45	0.234
Residual	44	17.0693	0.3879		
Total	59	33.7138			
Variate: Diameter	d.f.	s.s.	m.s.	v.r.	F. prob.
Replication	4	7.3799	1.8450	3.34	0.017
Contrast (Seedlings vs. Cuttings)	1	2.3862	2.3862	4.32	0.043
Contrast. Container type	4	9.3743	2.3436	4.25	0.005
Contrast. Plant age	2	1.1031	0.5516	1.00	0.376
Contrast. Container x age	4	5.5167	1.3792	2.50	0.056
Residual	44	24.2887	0.5520		
Total	59	50.0488			
Variate: Volume	d.f.	s.s.	m.s.	v.r.	F. prob.
Replication	4	0.0039139	0.0009785	6.01	<0.001
Contrast (Seedlings vs. Cuttings)	1	0.0002826	0.0002826	1.73	0.195
Contrast. Container type	4	0.0028620	0.0007155	4.39	0.004
Contrast. Plant age	2	0.0000426	0.0000213	0.13	0.878
Contrast. Container x age	4	0.0017356	0.0004339	2.66	0.045
Residual	44	0.0071667	0.0001629		
Total	59	0.0160035			

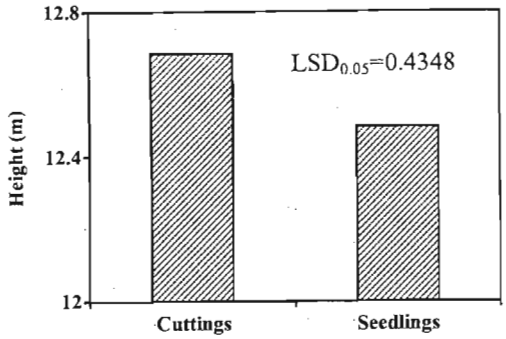


Figure 1. Mean tree height at seven years for the two plant types.

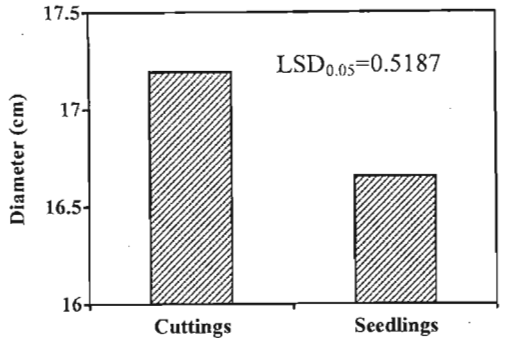


Figure 2. Mean tree diameter at seven years for the two plant types.

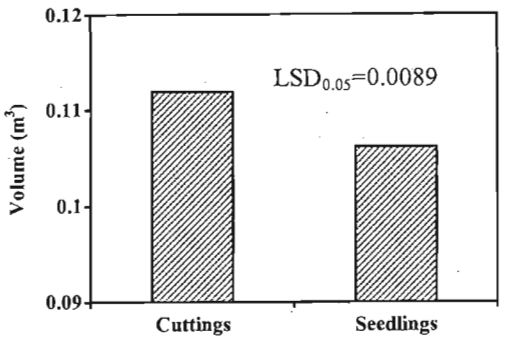


Figure 3. Mean tree volume at seven years for the two plant types.

The effect of tray type on growth was highly significant with the large (125 ml) Unigro 72 tray performing significantly better than all trays tested **(Table 5, and Figures 4, 5 and 6)**. The BCC tray was the poorest ranked tray treatment for diameter and volume growth. During the nursery-raising period a large portion of the growing medium fell out of the base of this tray reducing the effective root volume from 100 ml to approximately 60 ml. In addition, the many large slits in the side walls of the BCC tray, designed to air-prune lateral roots, resulted in periodic drying out of the growing medium. These two factors probably led to the poor field performance of cuttings raised in this tray type. The plant raising period in the nursery had no significant effect on height growth. However, there were interactions between tray type and age for diameter and volume growth **(Table 5)**. Although the interaction could not be explained, the older cuttings performed

better when grown in Sappi trays while the younger cuttings performed better when grown in Unigro or BCC trays **(Table 4)**.

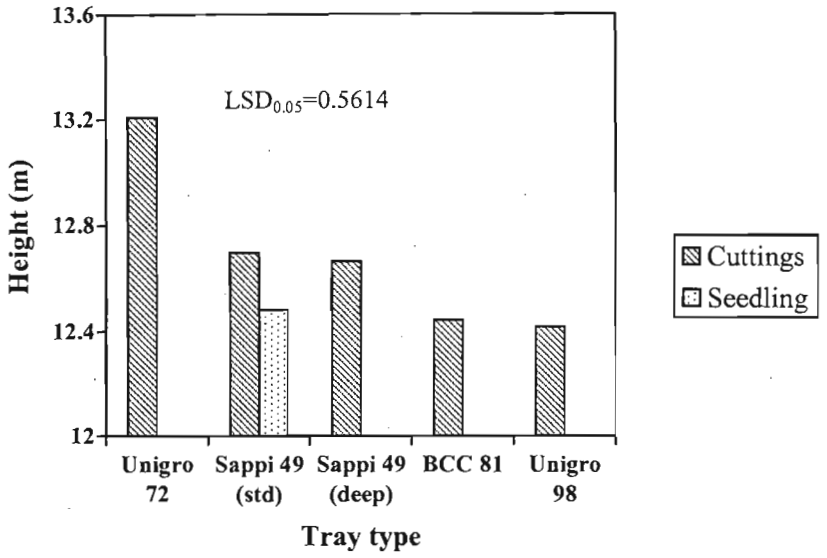


Figure 4. Mean tree height at seven years for the tray types tested.

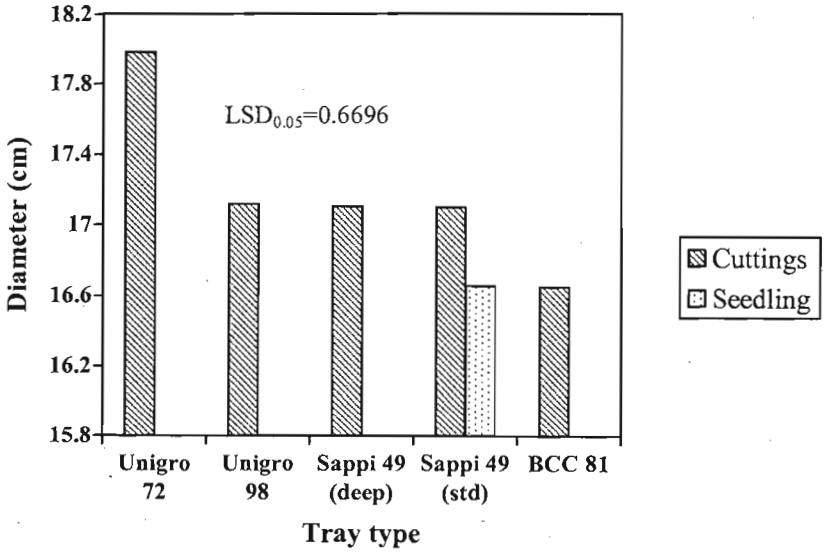


Figure 5. Mean tree diameter at seven years for the tray types tested.

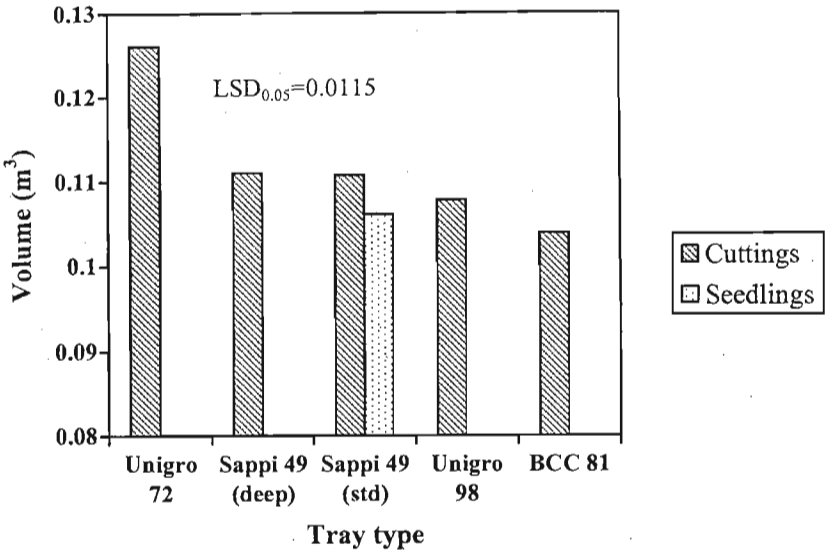


Figure 6. Mean tree volume at seven years for the tray types tested.

Linear regression analyses indicated that the volume of the root plug (media volume) and root mass at planting were the major contributors to the significant differences measured in height, diameter and volume at 7 years **(Table 6)**. The Unigro 72 (9-month-old) cutting treatment, which had the largest root mass at planting (0.560 g), yielded the second largest trees at 7 years. They were 27% larger in individual tree volume than those produced in the Sappi standard tray for a period of 7 months, which had the smallest root mass at planting (0.159 g) **(Table 3)**.

Exceptions to this general relationship were the Unigro 98, 9-month-old cuttings (0.459 RDM) and the Sappi deep tray (0.414 g RDM). Both performed relatively poorly in spite of their large root mass. The Sappi deep trays had cavities that were much longer and narrower than the Unigro 72 tubes (which produced the largest trees) and the most likely reason for the poorer performance could be that roots were damaged during the extraction process. It was noted at the time of planting that the plants grown in the Sappi deep trays were difficult to extract from the cavities due to excessive friction between the root plug and inner cavity wall.

Root collar diameter at planting correlated significantly with tree diameter and volume growth up to an age of 5 years **(Table 6)**, suggesting that this may be an important nursery variable to measure when establishing field trials. Height at planting had no influence on any field growth measurements. The observation that initial plant height had no effect on height growth, suggests that if genetic bias can be eliminated, height growth is controlled by other factors such as site quality (Bredenkamp, 1993). Furthermore, from the linear regressions carried out at each assessment, it was apparent that the influence of initial shoot dimension diminished over time. This indicates that, if shoot dimension at planting does influence early growth, it is likely to lose its influence at a later stage. Root description, therefore, is the most likely nursery morphological attribute contributing to field growth performance.

Table 6. The results of simple linear regression analyses on field growth responses due to nursery measurements at planting.

		2.5 years		5 years		7 years	
Nursery explanatory variate	Field dependant variate	F prob.	R^2	F prob.	R^2	F prob.	R^2
Height at planting	Height	0.139	0.13	0.654	0.00	0.902	0.00
	DBH	N/A	N/A	0.223	0.59	0.356	0.00
	Volume	N/A	N/A	0.356	0.00	0.535	0.00
Root collar diameter at planting	Height	**0.022**	0.37	0.135	0.13	0.613	0.00
	DBH	N/A	N/A	**0.032**	0.32	0.136	0.13
	Volume	N/A	N/A	**0.046**	0.28	0.319	0.09
Root dry mass	Height	**0.024**	0.39	0.124	0.16	0.289	0.03
	DBH	N/A	N/A	**0.017**	0.43	0.060	0.27
	Volume	N/A	N/A	**0.022**	0.40	0.111	0.18
Root volume	Height	**0.022**	0.36	0.106	0.16	0.065	0.23
	DBH	N/A	N/A	0.174	0.09	**0.049**	0.28
	Volume	N/A	N/A	0.112	0.16	**0.046**	0.28

Figures in bold indicate significant relationships at the 5% level

N/A = data not available

CONCLUSION

We believe that root volume (quantity of growing media) influenced tree height, diameter and volume up to 7 years after planting. It would appear that the reasons for improved field growth with larger root volumes are due to an increase in root mass. Treatments possessing a greater root mass at planting had the largest response on improving diameter, which in-turn, influenced tree volume. Cuttings possessing larger root collar diameters were found to correlate with larger diameter trees at 5 years of age. Plant height at planting had no relationship on medium-term field growth performance. From the data, it would appear that containerised cuttings should be raised in cavities at least 80 ml in volume and have attained a dry root mass of between 0.3 and 0.5 g for successful growth to be achieved after establishment. A well-colonised root plug with greater root mass was achieved by producing cuttings for a period of 9 rather than 7 months. Lastly it was encouraging to note that cutting survival and growth was similar to seedlings in this trial, indicating that the operational propagation of *P. patula* by means of cuttings is possible.

ACKNOWLEDGEMENTS

The authors wish to acknowledge field assistants T. Zulu, G. Malapane, R. Thibela and J. Phonela for carrying out the routine assessments, and the management forester, Mr. H. Pienaar, for his assistance in the silvicultural maintenance of the trial. Mr. J. Crous is acknowledged for his assistance with some of the statistical analysis. Mr R. Clausen, of Detectamatic c.c., is acknowledged for manufacturing the Sappi deep tray at no charge for the purpose of the trial.

CHAPTER V

A review on the effects of donor maturation on rooting and field performance of conifer cuttings

Mitchell, R. G., Zwolinski, J. and Jones, N. B., 2004. A review on the effects of donor maturation on rooting and field performance of conifer cuttings. Southern African Forestry Journal 201: 53 – 63.

ABSTRACT

The maturation and ageing effects of parent or donor plants have been reported to have both positive and negative influences on the performance of rooted cuttings. A general decline in rooting ability, root quality and speed of rooting in the nursery, and a reduction in tree survival, growth and form in the field, have been associated with donor plants that have reached a state of reproductive or ontogenetic maturity. Increased maturation has also been shown to affect wood quality negatively. Provided that donor plants are still relatively young, positive effects from increased donor age have been observed through an improvement in tree form and branching habit, as well as a reduction in bark thickness and stem taper. These improvements have resulted in increased timber yields over seedlings of the same genetic origin. This report summarises current understanding of the effects of donor maturation.

INTRODUCTION

Rapid multiplication of improved genetic material for the establishment of forest plantations is now commonly achieved through the production of rooted cuttings. The age of the parent material, used for the production of cuttings, has been found to be one of the most important factors affecting rooting (Girouard, 1973) with a decline in rooting ability with increasing hedge age. For this reason all plantation establishment by means of rooted cuttings is based on juvenile material. In addition to a decline in rooting, increased hedge age has also been known to affect post-planting survival, growth habits and wood quality. A good understanding of maturation effects and careful management of parent hedge material is, therefore, required to successfully produce rooted conifer cuttings that will yield satisfactory results.

BASIC TERMINOLOGY

Haffner *et al.* (1991) describes the process of maturation as the developmental process relating mainly to woody species, during which growth rate and rooting of cuttings is reduced by the onset of morphological changes in the parent plant. In plants there appear to be three separate, although highly interactive, forms of maturation, all of which are known to influence rooting and plant growth (Leakey *et al.*, 1992; Lewis, 2000). The first two forms represent true maturation and relate directly to the age of the donor plant (cyclophysis) and the position of the propagule on the donor plant (topophysis). The third element, is that of general donor plant health, and can be described as a carry over effect of the environment. This type of ageing is referred to as physiological ageing (periphysis) (Olesen, 1978).

The effect of donor age (cyclophysis)

Ontogenetic ageing

The term "ontogeny" is a broad term that describes "the developmental course of an organism from the fertilized egg through to maturity" (Oxford University Press, 2000). In horticulture, the term ontogenetic maturity refers to the onset of reproductive maturity (Leakey *et al.*, 1992). Ontogenetic ageing is due to epigenetic effects, or pre-programmed gene expression (Dekker-Robertson and Kleinschmit, 1991; S. J. Walker, Queensland Forest Research Institute, pers. comm., 2002; Allan, 2003). Although all parts of the plant may have identical DNA, or genetic makeup, different genes in the DNA are stimulated at different stages of development, which can result in variation in rooting, growth habit, flowering and leaf morphology. It is important to understand that the changes in rooting observed as the donor plant matures, is due to gene regulation, and not changes in the genetic makeup.

When we refer to age as one of the most significant factors influencing rooting in pines, we usually refer to the ontogenetic point at which plants would normally reach reproductive maturity for a given species (Slee and Clarke, 1991). It is also generally observed that mature trees grow at a slower pace, producing smaller annual growth increment rings, whereas during the juvenile phase, trees may grow continuously throughout the growing season or pass through repeated growth flushes (Borchert, 1976). Shoots, harvested from reproductively mature trees, exhibit poor rooting because they are produced from the previous year's growth where genes for good rooting and fast growth are no longer being expressed, but rather those for flowering and slower growth (S. J. Walker, Queensland Forest Research Institute, pers. comm., 2002).

There can be some exceptions to this rule. It has been shown that flowering can occur without the onset of irreversible maturation (Vieitez and Vieitez, 1976). In some cases, male strobili are the first to appear on pine trees (Giertych, 1976) and it has been found that rooting from shoots harvested from trees in the presence of male flowers, but in the

absence of female flowers is still acceptable (Giertych, 1976). Studies on *Pinus patula* in Zimbabwe, however, have revealed that female strobili are the first to appear and can appear on trees raised from seed as early as two years after planting (Barnes and Mullin, 1974).

Wilson (1998) warns against making a close association between a decline in rooting and onset of flowering and he speculates that rooting ability and flowering are under the control of two directly antagonistic hormones. He uses the example of *Eucalyptus deglupta* and *E. camaldulensis*, which are considered both easy to root and early flowerers, or *E. nitens*, which is considered difficult to root and flowers late. In a study carried out on *Pseudotsuga menziesii* (Black, 1972) rooting declined rapidly from trees older than 9-years and only 5% rooting was obtained from trees aged 24-years. Black concluded that the decline in rooting could not be attributed directly to the onset of flowering as only trees aged 24 and 25 years showed evidence of cones. Trees aged 15 years and less, carried no cones.

Chronological ageing

Chronological ageing refers to a purely linear description of age, categorized in terms of time. There are two forms of chronological age in trees. The first one categorizes the tree as a whole in terms of time e.g. a one-, five-, or ten-year-old tree. The second form of chronological age refers to differences in age on parts of the same tree. For example, deciduous trees produce new (chronologically young) leaves during spring and shed their leaves (chronologically old) during winter. This process occurs annually for the duration of the trees' lifetime.

Chronologically, the oldest part of any tree will be the portion of tissue laid down during the very early growth stages. Ontogenetically, the oldest part of any tree will be the portion of tissue laid down during last season's growth period. Annually, during each new growing season, new shoot development (chronologically youngest) is formed on the ontogenetically oldest part of the tree. It is therefore hypothesised that the shoots with a

juvenile appearance observed on the top parts of a tree that has reached reproductive maturity, will produce poor rooting, and shoots with a mature appearance observed on a young tree that has not reached reproductive maturity may produce good rooting.

The position of the propagule on the donor plant (topophysis)

A trend for increasing levels of maturity with height is common in addition to overall donor age (Roulund, 1975; Donald, 1987). This phenomenon is referred to as topophysis. It has been regularly observed that rooting from shoots harvested from the lowest branch whorls produce acceptable rooting whilst those harvested from the top of the crown are very difficult to root (Roulund, 1975; Donald, 1987). The reason for this is that trees grow from the base up and from the inner to the outer portions (Hamann, 1995) making the base region, ontogenetically, the most juvenile part of a tree.

Hedge condition (periphysis)

Physiological ageing

The loss of juvenility of the stock plant is a common description for any unexplained decline in rooting over a period of time (Leakey *et al.*, 1992). There are numerous sources of variation in rooting that are stock-plant related, but that have nothing to do with the loss of juvenility. However, because these effects are not properly understood, any decline in rooting due to them have been ignored and referred to as "phase change" or irreversible maturation (Leakey *et al.*, 1992). These changes are generally reversible provided ontogenetic maturation has not set in.

It has been shown that the physiological state of the donor plant is one of the most important factors in achieving acceptable rooting (Bower and van Buijtenen, 1977; Goldfarb *et al.*, 1997). This form of ageing refers to changes arising from variations in factors such as nutrient status, carbohydrate content and the water status of the donor plant and propagule (Leakey *et al.*, 1992). It has been determined that nutritional and

environmental stresses encourage biochemical changes associated with flower induction and the onset of early maturation (Durzan, 1976).

Interestingly, even with supplemental fertilizer, hedges receiving regular shoot removal can show increased nutritional deficiencies over time (Osorio, 1993). Nutrient deficiencies are particularly apparent from the third year of production. This has been observed in *Pinus oocarpa*, *P. maximinoi*, *P. tecunumanii* (Osorio, 1993), as well as in *P. patula* and *P. elliottii x P. caribaea* hybrid hedges (L. A. Williams, Sappi Forest Nurseries, pers. comm., 2003).

Conversely, accelerated optimal growth has also been known to induce maturation. In a study conducted on *Picea pungens*, seedlings were grown under a 24-hour photoperiod with optimal temperature, moisture and nutrient levels. The plants exhibited continuous and vigorous growth. Once planted, the trees demonstrated precocious flowering, compared to those grown under natural conditions in the nursery (Young *et al.*, 1976).

Preventing maturation

Maturation of many coniferous species of trees can be postponed significantly through various techniques such as hedge management, rejuvenation, manipulation of temperature and etiolation.

Hedging

This technique makes use of the effects of topophysis, and involves maintaining parent material in a hedged state where shoots can continually be formed from, or close to, the root collar area. In this way all new shoot material is formed from tissue that was laid down when the plant was producing juvenile tissue. The juvenile characteristics from this tissue are passed on to the newly formed shoots and rooted cuttings (Fielding, 1954; Reines, 1963; Fielding, 1969; St. Clair, *et al.*, 1985; Donald, 1987; Osorio, 1991; Copes, 1992; Haines *et al.*, 1992; Peer and Greenwood, 2001). This appears to be the most

successful and common technique employed by nursery managers today. As hedges grow in height, and shoots are subsequently formed from tissue possessing less juvenile characteristics, it may be necessary to annually "crash-back" or lower the height of the hedge closer to that of the original size (Libby *et al.*, 1972; Walker and Haines, 1991; Haines *et al.*, 1992).

Maximising the advantages that topophysis offer, some authors are using a technique called mini-cutting propagation (de Assis, 2001). This technique involves maintaining parent hedges in very small containers, and harvesting the shoot material just above the root collar. Often seedling trays are used for maintaining these hedges. Material harvested is no more than a few centimeters in length. It has been found that optimising hedge nutrition and water requirements with such hedges becomes increasingly important, however, as the small containers and effects of harvesting shoots so close to the root collar place increased stress on the hedge. Such stress may lead to premature physiological ageing and a decline in rooting.

Rejuvenation

A second, more complicated technique of delaying the effects of maturation involves what is commonly known as serial propagation or hedge cycling. This technique involves harvesting shoot material from donor plants that are reaching a point of ontogenetic maturation, and once rooted, establishing new donor plants from the rooted cuttings (St. Clair *et al.*, 1985; Goldfarb *et al.*, 1997). This process has been shown to significantly improve rooting ability of cuttings harvested from the newly established donor plants (Cameron, 1968; Goldfarb *et al.*, 1997). It has also been observed that cuttings harvested from parent hedges that are already showing signs of maturity through the change in foliage, once rooted produce more juvenile looking shoots with longer primary needles, shorter secondary needles and the presence of juvenile buds (Jacobs, 1939). Rejuvenation procedures on *Pseudotsuga menziesii* have shown to induce earlier bud-break (Black, 1972).

For the same reasons that the rooting ability of cuttings from cycled hedges is improved, the rooting ability from new shoots produced from grafted scion material, can be improved if scion material is grafted onto juvenile rootstock (Pawsey, 1971; Mahdi, 1985; Huang *et al.*, 1992; Osorio, 1993). The distance from the grafted scion to the base of the rootstock has also been shown to influence the degree of rejuvenation (Chaperon, 1979).

A promising method of attempting to rejuvenate mature trees is through repeated *in vitro* propagation. Propagation using *in vitro* methods is also known as micro-propagation due to the size of the plant material used. Studies show that rejuvenation occurs when buds are harvested from mature trees and placed on medium containing the cytokinin, Benzylaminopurine. These explants form new buds from developing needle fascicles (or short shoots). Attempts to rejuvenate *Picea sitchensis in vitro* (using apical bud tissue and callus formation from needles by subjecting the tissue to repeated cycles of cold storage at 2 °C and growth at 20 °C) resulted in cultures with a more juvenile character. Repeated sub-culturing of the callus formed from the needles increased the speed at which new callus was formed (Allan and O' Donnel, 2002).

Some authors believe that through repeated rejuvenation procedures and by propagation *in vitro*, ontogenetic patterns can be almost completely reversed in some species (Bonga and von Aderkas, 1993). Rejuvenation procedures, through repeated *in vitro* propagation, have been well documented for *Sequoia sempervirens*. In one study, trees aged 600 years showed visual signs of effective rejuvenation in the laboratory after 17 subcultures *in vitro* (Fouret *et al.*, 1985). Rejuvenation has also been observed through successive repeated grafting of adult shoot apices onto seedling rootstocks *in vitro*. In most cases 4-5 successive grafts were necessary to reinstall juvenile characteristics (Huang *et al.*, 1992; Mahdi, 1985).

Rejuvenation is believed to be due to a reduction in the distance from the shoot producing region and root collar, and change in the physiological conditions associated with a

recently formed root system (Hackett, 1985). A possible hormonal influence, as a result of a juvenile root system, has also been suggested (Jayawickrama *et al.*, 1991).

Reducing temperature

The third technique makes use of reduced temperature to slow down growth rate. This technique is used most commonly when attempting to reduce growth rate in the laboratory, whilst identifying desirable clones in field trials. During the field testing process representatives of clonal material are stored either as micropropagated shoots *in vitro,* at temperatures ranging between 2 and 6 °C, or by cryopreserving tissue in liquid nitrogen at –196 °C (Withers, 1984; Smith, 1986; Davies and Aitken-Christie, 1991; Allan, 2003). Trials conducted on cold-stored *P. radiata* shoot material, have shown that although much of the original material placed into cold storage is lost over time due to contamination (Davies and Aitken-Christie, 1991), shoot cultures can be stored up to 5,5 years with no adverse effects (Aitken-Christie and Singh, 1987).

Interestingly, a pretreatment of cold storage before setting has been observed to improve rooting. Libby and Conkle (1966) were able to improve the speed of rooting and increase the number of roots that formed per cutting, by subjecting cuttings to a cold treatment for a period 20 to 50 days before setting, and Brix (1973) reports an improvement in rooting if *P. radiata* cuttings are kept at 4,4 °C for a period of 6 weeks.

Etiolation

The degree to which plants are exposed to direct sunlight has also been shown to affect the juvenility of the donor plant. This effect, however, is probably more closely related to physiological condition rather than true maturation. Shaded donor hedges have been found to produce etiolated shoots with juvenile characteristics (Osborne, 1992). These shoots have been shown to produce better rooting and survival (Blakesley *et al.*, 1992). Studies on *P. patula* hedged plants (Osborne, 1992) show that the most heavily (85%)

shaded hedges produced cuttings that rooted significantly better and produced the highest average root length above all other shading treatments.

OBSERVATIONS

Nursery performance

Rooting efficiency

A summary of the rooting studies of coniferous plants examined in this review is given in **Table 1**. The rooting percent varied from 25% to 100% for juvenile material and from 1% to 75% for mature material depending on species and external factors that may have affected the outcome of the studies. Rooting efficiency from juvenile hedge material in all cases appeared acceptable for large-scale cutting production. The biggest limiting factor of some species seemed to be the early maturation age. This was particularly true of *P. patula*, *P. pinaster* and *Larix spp.* **(Table 1)**.

As mentioned under the description of ontogenetic maturation, a decline in rooting efficiency does not necessary provide an accurate measure of irreversible maturation however. In a study conducted on the nursery and field performance of *P. taeda* cuttings and seedlings, cuttings from 5-year-old tree donors rooted equally well compared to those from 1-year-old tree donors and had a similar quality root system. The cuttings from the 5-year-old donors, however, were found to grow significantly slower in the field after planting (Foster *et al.*, 1987).

Table 1: A summary of the published information reviewed on maturation age and rooting ability of coniferous species of trees

Species	Reported maturation age (without rejuvenation)*	Reported maturation age (with rejuvenation)**	Rooting percent from juvenile donors	Rooting percent from mature donors	References
Chamaecyparis nootkatensis	7 years [1]	10 years [2]	90% (5-year-old hedges) [2]	62% (12-year-old hedges) [2]	1. Karlsson and Russell, 1990 2. Russell and Grosnickle, 1989
Larix spp.	1 year [1]	N/R	N/R	N/R	1. Peer and Greenwood, 2001
Picea abies	12-16 years [3, 5]	15-21 years [2, 4]	97% (14-year-old-hedges) [1] 100% (Juvenile seedling donors) [5]	50-75% (12-year-old trees) [5]	1. Bentzer, 1988 2. Dekker-Robertson and Kleinschmidt, 1991 3. Roulund, 1975 4. St. Clair *et al.*, 1985 5. Von Wühlisch, 1984
Pinus banksiana	4 years [1]	N/R	74% (4-year-old trees) [1]	18% (30-year-old trees) [1] 45% (5-year-old trees) [1]	1. Browne *et al.*, 1996
Pinus caribaea	4 years [1]	N/R	90% (Juvenile seedling hedges) [1]	N/R	1. Dvorak *et al.*, 2000 (b)
Pinus chiapensis	More difficult than *P. maximinoi*, *P. patula* and *P. tecunumanii* [1]				1. Dvorak *et al.*, 2000 (c)
Pinus elliottii	N/R	N/R	19-63% (4-year-old hedges) [1] 77% (Juvenile seedling hedges) [2]	N/R	1. Bower and van Buijtenen, 1977 2. Frampton and Hodges, 1989
Pinus elliottii x Pinus caribaea	N/R	N/R	>80% (<3-year-old hedges) [2] 90% (Juvenile seedling hedges) [1]	N/R	1. Haines and Nikles, 1987 2. Walker and Haines, 1991
Pinus greggii	*P. greggii* var. *greggii* is easier to propagate than var. *australis* and both are easier to propagate than *P. patula* [1]				1. Dvorak *et al.*, 2000 (d)
Pinus herrerae	N/R	N/R	86% (Juvenile seedling hedges) [1]	N/R	1. Dvorak *et al.*, 2000 (e)
Pinus jaliscana	N/R	N/R	65% (Juvenile seedling hedges) [1]	N/R	1. Dvorak *et al.*, 2000 (a)
Pinus maximartinezii	N/R	N/R	70% (Juvenile seedling hedges) [1]	N/R	1. Dvorak *et al.*, 2000 (f)
Pinus maximinoi	N/R	N/R	64% (4-year-old hedges) [2] 82-88% (1-year-old hedges) [1]	N/R	1. Osorio, 1991 2. Osorio, 1993
Pinus oocarpa	N/R	N/R	N/R	41% (5-year-old trees) [1]	1. Easley and Lambeth, 1989
Pinus patula	N/A	2-3 years [1]	>80 (1-year-old hedges) [1]	65% (3-year-old hedges) [1]	1. Mitchell, unpublished
Pinus pinaster	Difficult due to rapid ageing [1]				1. Clarke, 1992
Pinus radiata	5-6 years [1, 3, 6, 7]	8 years [2, 5]	70% (2-year-old trees) [4] 80% (<5-year-old trees) [6] 88% (3-year-old trees) [3]	11% (26-year-old trees) [3] 40% (9-year-old trees) [4] 68% (5-year-old trees) [3]	1. Cameron and Thomson, 1969 2. Clarke and Slee, 1991 3. Fielding, 1954 4. Libby and Conkle, 1966 5. Libby *et al.*, 1972 6. Menzies *et al.*, 1985 7. Sweet and Harris, 1976
Pinus taeda	4 years [1, 2, 3, 5]	7 years [4]	25-95% (3 to 7-year-old hedges) [4] 45% (1-year-old trees) [1] 69% (Juvenile seedling hedges) [6]	28% (5-year-old trees) [1]	1. Foster *et al.*, 1987 2. Foster, 1988 3. Frampton and Hodges, 1989 4. Greenwood and Nussbaum, 1981 5. Greenwood, 1984 6. Hamann, 1995
Pinus tecunumanii	N/R	N/R	56-76% (1-year-old hedges) [1] 80% (Juvenile seedling hedges) [3]	54% (4-year-old hedges) [2]	1. Dvorak *et al.*, 2000 (g) 2. Osorio, 1991 3. Osorio, 1993
Pseudotsuga menziesii	8 years [2, 3, 4, 5, 7]	25 years [7]	70% (9-year-old trees) [7] 74% (25-year-old hedges) [7] 90% (7-year-old hedges) [3] 95% (<3-year-old trees) [6]	15% (80-year-old trees) [6] 25% (50-year-old trees) [1] 40% (12-year-old trees) [3] 45% (14 to 20-year old hedges) [2] 47% (10 to 13-year-old trees) [7] 50% (45-year-old hedges) [1] 60% (12-year-old trees) [3]	1. Bhella and Roberts, 1975 2. Black, 1972 3. Brix, 1973 4. Copes, 1992 5. Haemann and Owens, 1972 6. Maschke and Weiser, 1988 7. Roberts and Moeller, 1978

* = Cuttings were harvested from tree donors established from seed

** = Cuttings were harvested from donors that had been kept in a juvenile state through hedging or a combination of hedging and serial propagation

N/R = Not reported (no data obtained)

Root initiation

It has also been established that the speed and method of root initiation varies with increasing donor plant age. It is generally accepted that cuttings harvested from older hedges take longer to root (Libby and Conkle, 1966; Foster, 1984). Studies on *P. radiata* indicate that root formation from juvenile shoot material originates directly from single parenchyma or resin duct cells (Smith and Thorpe, 1975) while root formation from shoots harvested from mature donor plants develop from a callus arising mainly from the cortex (Cameron and Thompson, 1969). This indicates that as hedge donor plants mature, callus formation may increase before root primordia develop. Bhella and Roberts (1975) established that root initiation in *Pseudotsuga menziesii* arose from a callus mass consisting of loosely arranged parenchyma cells produced mainly in the vascular cambium, and to a lesser extent, from phloem and xylem. In this study, no differences in the origin and method of root initiation were observed between cuttings from different aged donors. The youngest material used, however, was a 7-year-old sheared tree. Increased callus production may also be as a result of other factors. Osorio (1993) reports that up to 25% of cuttings that appear to have rooted in the rooting environment, develop a callus without any root primordia and that this may be a physiological response due to unfavorable propagation conditions.

Root quality

In addition to a general decline in rooting ability and length of time that it takes to root with age, the quality of the root system, as measured by root dry mass and the number and length of roots, also decreases (Libby and Conkle, 1966; Menzies *et al.*, 1985; Dooley, 1986; R.G. Mitchell, Sappi Forests Research, unpublished results). In one study, the root quality of *Larix* cuttings was reported to decrease with increasing hedge age more rapidly than the decline in rooting percent (Peer and Greenwood, 2001). Cuttings with a poor quality root system, and poor plant condition, result in poor growth and

survival after planting (Jacobs, 1939; Fielding, 1954; Foster *et al.*, 1986; Ritchie *et al.*, 1993).

By adjusting the rooting environment, however, some studies have shown that acceptable plant quality can be achieved from donor hedges already showing signs of maturation. Trials on *Chamaecyparis nootkatensis* established that acceptable rooting could be obtained from older hedges by leaving cuttings for longer to root in the greenhouse and by using bottom heat to assist with the rooting process (Russell and Grossnickle, 1989). Brix (1973) also found that rooting of cuttings from mature *Pseudotsuga menziesii* significantly improved when the root zone was heated. Reports indicate that cuttings raised from older hedges in this manner can produce good quality plants that grow as well as those from the more juvenile hedges (Wise, 1994). It has been observed, however, that shoots from older hedges are more susceptible to attack by pathogens in the rooting environment (Osorio, 1993).

Root pruning of bare rooted cuttings from older hedges has also shown to increase the number of roots per cutting base and an overall improvement in root quality. If roots are removed from the callus base, new roots are formed which would not have been formed had the original roots been left intact (Cameron, 1968). Cameron (1968) believes that the reasons behind this phenomenon are due to the fact that actively elongating roots produce auxins that inhibit the production of new roots, and any new root development that may be observed after planting out may be as a result of the intended, or accidental, root pruning that occurs at planting.

Field performance

Survival

Conflicting findings are reported regarding an increase in donor age and field survival of *P. radiata* cuttings. Most studies indicate a negative effect of age on survival (Fielding, 1970; Libby and Hood, 1976; Bolstad and Libby, 1982). Own trials conducted on

P. patula have yielded differing results. One out of four trials showed that cutting survival declines with increasing hedge age, while three trials indicate better survival from older hedges (R.G. Mitchell, Sappi Forests Research, unpublished results). It was noticed that as hedge age increased in these trials, cuttings appeared more lignified and less succulent. Comparative survival between cuttings, that are naturally more mature, and seedlings in *P. radiata* (Cameron *et al.*, 1987; Ades and Simpson, 1990) and *Picea abies* (Hannerz, 2003) indicate significantly better survival with cuttings under drought and extreme cold conditions. In these studies, the cuttings were reported to be sturdier than seedlings, possessing thicker collar diameters, a larger, more vigorous root system (if grown in a bare-rooted manner) and a more lignified stem. Better survival, from cuttings harvested from older hedges, may therefore be due to these morphological differences and not due to an increase in donor age and the effects of ontogenetic ageing.

Susceptibility to pests and pathogens

Studies on the properties of the wax on the surface of *P. radiata* needles have shown that the chemical composition of the wax changes with age. Furthermore, in old trees, the stomata can become blocked (Wells and Franich, 1977). This is thought to prevent penetration by the needle blight fungus, *Scirrhia pini* and accounts for the widespread susceptibility in young trees to virtual immunity in older (18-year-old) trees (Anon., 1977). Studies in *P. radiata* show that an increase in donor maturation is correlated with increased resistance to *Dothistroma septospora* (Garcia and Kummerow, 1970; Ivory, 1972; Burdon and Bannister, 1985; Ades and Simpson, 1990) and *Endocronartium harknessii* (Zagory and Libby, 1985; Power *et al.*, 1994). Increased donor maturation has also resulted in increased resistance to *Cronartium quercuum* in *P. taeda* (Frampton *et al.*, 2000).

Tree form and shape

It appears that some maturation is beneficial in terms of improving tree form. Studies on *P. radiata* indicate that cuttings from juvenile donor hedges produce fewer, shorter and smaller branches, without any apparent loss in tree volume (Menzies and Klomp, 1988; Faulds and Dibley, 1989). Cuttings harvested from tree-origin donors have been found to produce better form with less taper, and fewer and smaller branches compared to hedge-origin donors (Bolstad and Libby, 1982). Scion material, from 12-year-old *P. radiata* tree-donors, have shown to produce trees with less branching and up to 80% less needle dry matter, than scion material from 1-year-old tree-donors (Greenwood, 1984). Compared to trees grown from seed, cuttings from more mature donors produce primary branches at a more perpendicular angle to the main stem (Bolstad and Libby, 1982). It has also been reported that cuttings, from juvenile donors, have less butt-sweep and stem crookedness than seedlings (Burdon and Bannister, 1985; van der Sijde and Roelofsen, 1986). Studies on *Chamaecyparis nootkatensis* seedlings, and cuttings from 1-, 3- and 7-year-old donors, have revealed that cuttings produce fewer multiple leaders compared to seedlings (Karlsson and Russell, 1989).

As donor plants, whether in hedge or tree form, reach ontogenetic maturation, however, cuttings harvested from them produce malformed stems (Roulund, 1975; Peer and Greenwood, 2001). Trees, whilst still in a juvenile state, will continue to produce new leaders that will replace the main leader if it is removed from the tree during its stage of growth. However, once the tree reaches the point of ontogenetic maturation, removal of the main leader will not stimulate it's replacement by a branch. The tree will continue to grow without any change in orientation. This effect, observed by the horizontal growth form of the lateral branches, is referred to plagiotropism and cuttings harvested from parent material that have reached an irreversibly mature state may express plagiotropic growth in the field (Roulund, 1975; Dekker-Robertson and Kleinschmit, 1991; Peer and Greenwood, 2001).

In many cases, cuttings harvested from recently matured donors continue to display plagiotropic characteristics during the early stages of field growth but display vertical growth habits, otherwise known as orthotropic growth, after a few years. It is believed that the change in growth habit from plagiotropic to orthotropic could be as a result of the production of compression wood (Frampton *et al.*, 2000). Compression wood is a response to gravity and stress and is known to have a lower cellulose and higher lignin content (Butterfield and Meylan, 1980). It is viewed as a serious defect in timber due to its weaker structure. Cuttings harvested from donors in the advanced stages of ontogenetic maturation, however, do not regain orthotropic growth patterns. A study on *Picea abies* revealed that after 4 years in the field, cuttings from donors aged 6-13 years had changed to orthotropic growth and those from donors aged 16-20 years continued to display plagiotropic growth patterns (Roulund, 1975). By serially propagating *P. abies* every 3 years for a period of 16 years (5 cycles), no plagiotropic growth patterns were observed in the field after planting, indicating that juvenility had been maintained through repeated serial propagation (St Clair *et al.*, 1985). Provided maturation is not too severe, improvements in growth habit can occur fairly promptly. Changes from plagiotropic to orthotropic growth patterns were observed in *Pseudotsuga menziesii* cuttings during their nursery-raising period (Ritchie, *et al.*, 1994), and in *P. taeda* cuttings, from 2,5-year-old hedge donors, soon after planting out (Frampton *et al.*, 2000).

Growth rate

In addition to the benefits of improving stem form and branching habits, cuttings harvested from juvenile donor plants appear to grow as well as seedling controls producing similar over-bark volumes. Trees from mature donors do not grow as fast, however. The first indication that cuttings have been harvested from mature donor plants can usually be seen by a reduction in stem diameter (Bolstad and Libby, 1982; Menzies and Klomp, 1988; Menzies *et al.*, 1991) but due to the improved stem form, taper and reduction in bark thickness, however, the total utilizable volume may be greater than that for seedlings at rotation age. In a destructive sampling study (Penman, 1988), it was established that although *P. radiata* trees established from cuttings from 7-year-old

parents were thinner (over-bark) than trees established from seedlings, there was an overall 8% improvement in utilizable timber from the cuttings. A reduction in bark thickness from cuttings has been reported elsewhere (Klomp and Hong, 1985). Penman (1988) concludes that different tree volume and taper equations are needed for seedlings and cuttings.

A study on *P. radiata* (Libby and Hood, 1976) compared the growth of cuttings from 7-year-old hedges to that of the same aged tree-origin donors. In the study, it was established that cuttings from the hedge-origin donors produced longer needles and more branches than those from the tree-origin donors. Tree-origin donors were found to produce more bole-volume growth per needle length however, which suggests that cuttings from more mature donors allocate more photosynthate to bole per unit leaf area (Libby and Hood, 1976).

Wood quality

Studies on *P. radiata* cuttings show a decrease in wood density and shrinkage on air-drying with increasing donor age (Nicholls *et al.*, 1976; Sweet and Harris, 1976; Burdon and Low, 1991). A decline in wood density with age has also been observed in *P. oocarpa* cuttings (Osorio, 2000). Conversely, an increase in tracheid length, spiral grain, pith diameter and extractive content has also been reported with increasing age (Nicholls *et al.*, 1976; Sweet and Harris, 1976). In the study carried out by Sweet and Harris (1976), significant clonal variation was observed, and the authors conclude that problems such as declining wood density with age could be reduced by screening clones.

CONCLUSION

The process of maturation in coniferous tree species is a complex process that affects the performance of rooted cuttings both negatively and positively. It appears that it is predominantly the effects of donor age (cyclophysis) and position of the propagule on the

donor plant (topophysis) that can be regarded as having an influence on the true maturation state of propagules as measured by rooting frequency, root system quality and field performance. The effects of the environment (periphysis) on donor plants are also known to influence the performance of propagules in the nursery and after planting but are often confused with cyclophysis and topophysis as the observed effects are very similar.

Various techniques of arresting the maturation process have been reported. The most common technique is to maintain the donor plant in a "hedged" state, approximately 15 cm high, in order to stimulate shoot production from the juvenile root collar region of the stool plant. A second technique, often used in conjunction with hedging, is that of serial propagation. This technique involves harvesting shoot material from donor plants and establishing new donors from the rooted cuttings. This technique is also known as cycling or cascading and it is reported that the material undergoes a process of rejuvenation during each cycle.

Many reports cite the most negative influence of increasing donor age as a decline in rooting and general nursery performance. It appears that the effects of increased donor maturation are first observed in the nursery. In one study conducted on the nursery and field performance of *P. taeda* cuttings, however, cuttings from 5-year-old tree-donors rooted equally well compared to those from 1-year-old tree-donors, but grew significantly slower in the field after planting (Foster *et al.*, 1987). There were reports of methods used to overcome nursery problems associated with maturation. These included the use of bottom heat to stimulate rooting, leaving the cuttings to root for a longer period in the nursery and root wrenching bare-rooted cuttings in order to produce a more fibrous root system.

The factor that has the greatest influence on cutting production is the age at which the donor plant reaches reproductive maturity and is most commonly termed, the point of "ontogenetic maturation". Once a donor plant had attained this state, manipulation of the donor to regain juvenile characteristics once more is very difficult, although through

repeated serial propagation, some authors achieved a degree of juvenility. Once this state has been reached, cuttings harvested from ontogenetically mature donors, display plagiotropic (malformed) and reduced growth habits. Field performance of cuttings derived from donor-plants that have not yet reached a state of ontogenetic maturity, however, may surpass that of seedlings due to better stem form, thinner bark and decreased taper. All the reviewed effects of increasing donor maturation on nursery and field performance have been summarised in **Table 2**.

Table 2. Summary of the relationship between hedge age and those factors important in conifer cutting production, establishment and growth

Factors assessed		Reported effect
1. Nursery performance		
1.1.	Rooting percent	Decreases with maturity
1.2.	Speed of rooting	Decreases with maturity
1.3.	Root number	Decreases with maturity
1.4.	Root mass	Decreases with maturity
1.5.	Callus formation	Increases with maturity
1.6.	Time to bud break	Increases with maturity
2. Field performance		
2.1.	Survival*	Decreases with maturity
2.2.	Pest resistance	Increases with maturity
2.3.	Growth rate	Decreases with maturity
2.4.	Growth form	Improves initially, then declines
2.5.	Taper	Decreases with maturity
2.6.	Branch thickness	Decreases with maturity
2.7.	Branch quantity	Decreases with maturity
2.8.	Branch angle	Increases with maturity
2.9.	Crown mass	Decreases with maturity
2.10.	Bark thickness	Decreases with maturity
3. Wood quality		
3.1.	Density	Decreases with maturity
3.2.	Tracheid length	Increases with maturity
3.3.	Spiral grain	Increases with maturity
3.4.	Shrinkage	Decreases with maturity
3.5.	Extractives	Increases with maturity
3.6.	Pith diameter	Increases with maturity

* In most cases a negative relationship existed between increasing donor age and survival although some authors reported a positive relationship.

ACKNOWLEDGEMENTS

The authors would like to thank Teresa Coutinho of the Tree Pathology Cooperative Programme at the University of Pretoria, and Ross Allan and Arnulf Kanzler of Sappi Forests Research, for proof reading this manuscript before submission.

Chapter VI

The effects of ontogenetic maturation in *Pinus patula* - Part I: nursery performance

Mitchell, R. G., Zwolinski, J. and Jones, N. B., 2005. The effects of ontogenetic maturation in Pinus patula – Part I: nursery performance. Southern African Forestry Journal 202: 29 – 36.

ABSTRACT

The age at which parent or donor hedges reach ontogenetic maturity has frequently been cited as a debilitating factor in the production of conifer cuttings. This point varies between species and prevailing environmental conditions. Among other things, a lack of knowledge of the effects of hedge maturation in *Pinus patula* has resulted in reluctance among South African foresters to plant cuttings of this species. Consequently, several trials were established between 2000 and 2003 to investigate the effects of ontogenetic maturation on the performance of *P. patula* cuttings in the nursery and field. This paper forms the first component of a three-part series and reports on those effects observed in the nursery. The effects of hedge maturation on field performance and cycling of *P. patula* hedges as a means of rejuvenation are reported separately. An analysis of the nursery data indicates that rooting efficiency, root system quality, and stem size and form, all decline with increasing hedge age. A decline in root system quality was particularly apparent and was observed prior to a decline in rooting efficiency.

INTRODUCTION

Pinus patula is the most important softwood species in South Africa. It has historically been propagated from seeds harvested from numerous open pollinated family trees selected for improved growth and stem form. Seed is usually bulked together before sowing and sown as a "family mix", and the improvement expected over unimproved material, can be calculated by averaging the yield improvement of all families making up the mix. Yield gains can further be improved by refining the selection process through selecting only top performing families, and dispatching seedlings on a per family basis (Kanzler, 2004). This method, however, restricts the quantity of seedlings available for dispatch making it impractical for large-scale operations. A common technique employed to overcome such difficulties is vegetative propagation of superior families through the production of cuttings. One of the factors most limiting to the success of this method of conifer propagation is the age at which donor plants mature. Hedge maturation refers to the initiation of morphological changes in the mother plant, which in turn affect the growth rate and rooting ability of cuttings (Haffner *et al.* 1991). The impact of this phenomenon is known to vary between species and investigation has already shown that maturation may occur particularly early in *P. patula* (de Jager, 2000). Extensive research efforts are therefore required before vegetative propagation can be employed to mass-produce cuttings from superior families for commercial deployment. This paper describes the effects of ontogenetic maturation on rooting efficiency, root system quality, and stem size and form, in *P. patula* cuttings during the nursery-raising period.

MATERIALS and METHODS

Between 1998 and 2001, seed was sown for the establishment of hedged plants to determine the effect of hedge maturation on tree performance in several nursery and field trials. Hedges were therefore raised to represent different age classes. Nursery data for three of these trials are reported herein. For convenience, the trials are referred to as Trials 1 to 3 **(Table 1)**.

Table 1. A summary of the trials: assessment dates, treatments, and characteristics of the planting stock before planting (stem size and form) or after 45-day pot studies (root mass and number)

Trial No.	Assessment Date	Treatments	Characteristics
1	April 2000	• Seedling • 12-month-old hedge • 24-month-old hedge • 36-month-old hedge	• Stem size • Root dry mass • Root numbers
2	April 2002	• Seedling hedges over a 48-month-old nursery period hedge	• Rooting efficiency
3	March 2003	• Seedling • 8-month-old hedge • 20-month-old hedge • 44-month-old hedge	• Stem size • Stem form • Root dry mass • Root numbers

Raising the plant material

Hedges

Plant material, selected for hedge establishment, was obtained as seeds from open pollinated families. Between 2 and 3 families were selected for each trial. When the seedlings were six-months-old, they were established in well-composted pine bark in 10 l black plastic bags, and kept in a semi-sheltered part of the nursery under 30% shadecloth. In their seventh month, they were cut back to a height of 15 cm to establish hedges. New shoots were selectively harvested every 4 to 6 weeks thereafter, depending on the season and hedge age. Irrigation was applied as required depending on rainfall. Hedges were fertilised monthly with a N:P:K water-soluble fertiliser (2:3:2 (42)) combined with 2 l of water per bag at a rate of 1%.

Cuttings

In all cases, cuttings were set into moist, well-composted and non-enriched, pine bark without the application of a rooting hormone. The rooting medium was first sieved through a 12 mm round screen to remove oversized bark particles. In all trials, the "Sappi 49" round-cavity white tray was used with individual cavity volumes of 80 ml. Before setting, shoots harvested from all hedges of the same age class and family were bulked together and thoroughly mixed. Once set, cuttings were placed in a fiberglass greenhouse where they received intermittent mist to prevent desiccation, and where bottom heat was applied in order to maintain media temperatures above 25 °C for the duration of the rooting period. Cuttings were left to root for a period of 60 to 90 days, depending on the season, before being removed and placed under 30% black shadecloth on raised beds where they received routine irrigation as required. Weekly fertiliser applications alternated between N:P:K water-soluble fertilisers in the ratio of 2:3:2 (42) and 5:2:4 (43) at a rate of 1%.

Seedlings

Seedling controls, raised for comparative purposes, were sown in the same tray type as those used for the cuttings, and under the same irrigation and fertigation regime. All seedling controls were of the same genetic stock as that of the cuttings. Seedlings were grown adjacent to the cuttings in the nursery beds during the raising period. Due to the increased time required to raise cuttings compared to seedlings, the seedlings were sown approximately 1 month after the cuttings had been set for each trial.

Assessing the planting stock

Plant assessments in the nursery were carried out as close as possible to the time of field establishment. These were conducted on a separate sample of plants, which included the cuttings derived from hedges of various ages as well as the seedling controls (Trial 1 and

3). The trials were assessed for rooting efficiency, root dry mass, the number of roots produced, and stem size and form **(Table 1)**.

Rooting efficiency

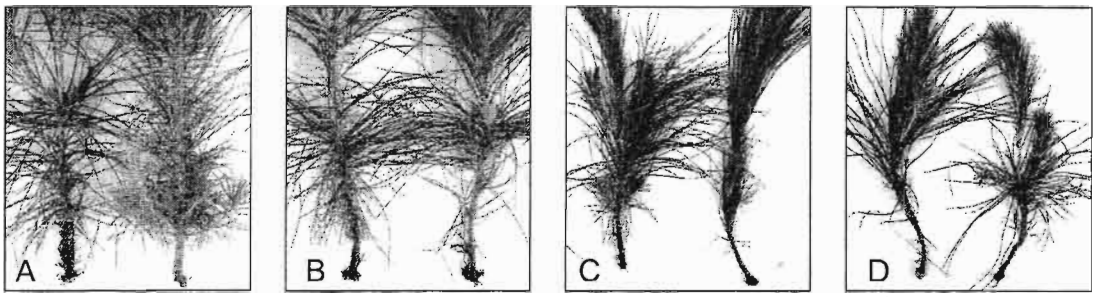
In Trial 2, rooting efficiency was assessed over a period of 42 months from the date of hedge establishment (48 months from sowing) by calculating rooting percentage at each setting. Hedges were harvested between 7 and 10 times each year.

Root development

In Trials 1 and 3, a sub-set of plants per treatment (20 in Trial 1 and 30 in Trial 3) were planted, each in 1 l bags, to assess root growth over a 45-day period. Plants were carefully removed from the bags after this period and all growing medium was washed from the root plug under running water. The number of roots produced from each individual plant was then counted and carefully removed from the cutting base, or root collar region in the case of seedlings, by using a sharp blade. Then, roots were placed into a labeled envelope for defining their mass after drying at 70 °C for 48 hours.

Stem size and form

Prior to field planting, plants in Trials 1 and 3 were assessed for root collar diameter (RCD) (using digital callipers) and stem height (Ht) by measuring from the base of the stem (in line with the medium surface) to the apical bud. From these values, Biomass Index (BI) was calculated using the formula $BI = RCD^2 \times Ht$ (mm) as an indication of plant size. Cuttings and seedlings were assessed for stem form, using a separate sample of plants to investigate whether plagiotropic growth patterns were apparent in shoots from older hedges. This was only conducted for the treatments in Trial 3. Stem form was scored according to four categories: 4 = Excellent, 3 = Good, 2 = Poor, and 1 = Very poor **(Figures 1 A-D).**



Figures 1 A-D. Stem form at planting. A. Excellent, B. Good, C. Poor, and D. Very poor.

Statistical analysis

All data was subjected to an analysis of variance (ANOVA), by using Genstat 5, version 3.2® (Genstat, 1995), to analyse differences between means. All least significant difference values (LSD) reported are based on a 5% significance level. All percentage data was subjected to an arcsine transformation prior to analysis (Zar, 1984). Due to the unbalanced nature of the rooting data, contrasts were used on the transformed rooting percentages comparing the rooting obtained in years two, three and four, with that in year one. All categorical data (number of roots and stem form values) were subjected to a square root transformation prior to analysis (Zar, 1984). Graphical presentation of this data is based on untransformed means. Different indices indicate significant differences between treatments. A regression analysis, performed on the numbers of roots and root collar diameters, was conducted after the number of roots were transformed using the logarithmic transformation (Zar, 1984). Due to the large amount of data available from the three trials reported on, family information was pooled in each case.

RESULTS and DISCUSSION

Rooting efficiency

The comparisons in rooting between years 2, 3 and 4, with year 1, revealed a significant decline in rooting from hedges age three years or older **(Table 2)**. There was a decline in

the average rooting percent per year, since the hedges were established **(Figure 2)**. This observation is consistent with reports on rooting efficiency and donor plant age in other conifer species (Black, 1972; Girouard, 1973; Haffner *et al.*, 1991; Peer and Greenwood, 2001). A decline in rooting efficiency is usually cited as the first indication of the onset of ontogenetic aging (Dekker-Robertson and Kleinschmidt, 1991) and is usually referred to as the point at which plants would normally reach reproductive maturity (Slee and Clarke, 1991). Support for the evidence that maturation occurs early in *P. patula* has been observed in field flowering studies where trees, raised from seedlings, produced cones as early as two years after planting (Barnes and Mullin, 1974). It is furthermore disturbing to note that, although the hedging technique used in this study is known to postpone the effects of maturation in other conifer species (St. Clair, *et al.*, 1985; Peer and Greenwood, 2001), hedging appeared to have had little impact on prolonging the maturation period beyond the age that Barnes and Mullin (1974) observed flowering in young trees.

Table 2. An ANOVA table showing the contrasts performed on the transformed rooting percentage data obtained from Trial 2.

Source of variation	d. f. (m.v.)	s. s.	m. s.	v. r.	F pr.
Year	3	13419.0	4473.0	27.09	<.001
Contrast 4 vs. 1	1	11924.6	11924.6	72.22	<.001
Contrast 3 vs. 1	1	1117.6	1117.6	6.77	0.010
Contrast 2 vs. 1	1	69.6	69.6	0.42	0.517
Residual	128 (2)	21135.6	165.1		
Total	131 (2)	33191.6			

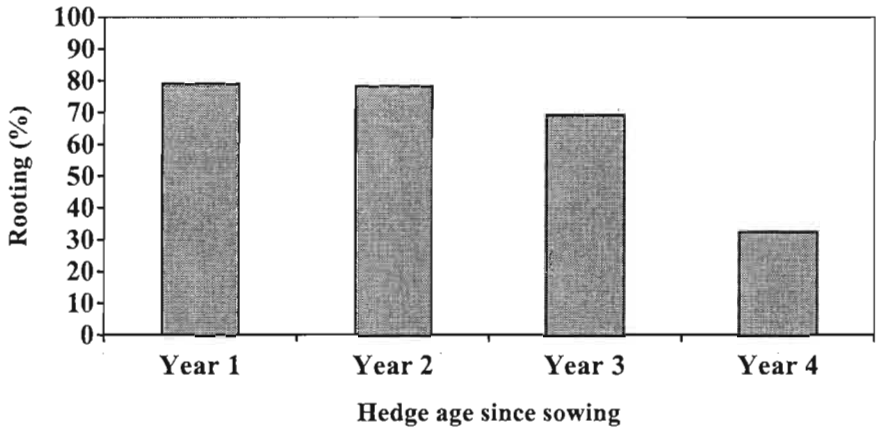


Figure 2. Mean annual rooting percent of cuttings harvested from seedling hedges over a 48-month period in Trial 2.

Root quality

Root architecture

From the assessment carried out on the sample of plants that were bagged up (Trials 1 and 3), the number of roots produced declined significantly with increasing hedge age **(Figures 3 and 4)**.

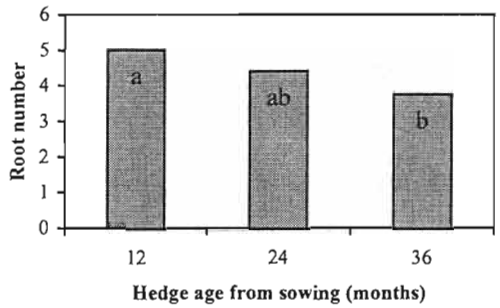


Figure 3. The number of roots per cutting from various-age hedges in Trial 1. Treatment means significantly different from one another (based on transformed values) are indicated by a different letter ($p<0.05$).

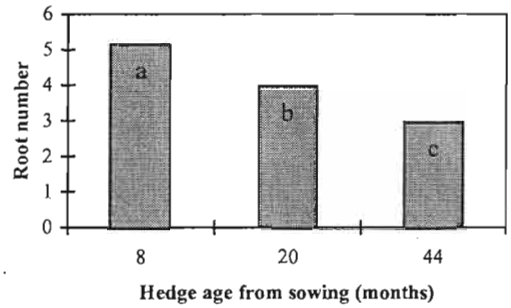
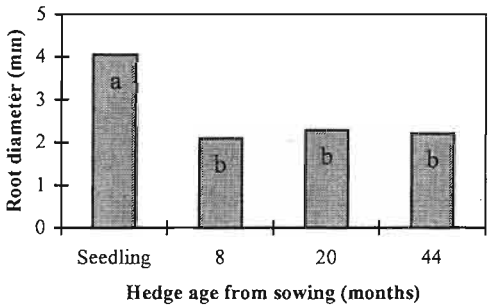


Figure 4. The number of roots per cutting from various-age hedges in Trial 3. Treatment means significantly different from one another (based on transformed values) are indicated by a different letter ($p<0.05$).

Differences in average root diameter at the point of emergence from the callus were insignificant for the cutting treatments **(Figure 5)**. This is most likely explained by the significant negative relationship that existed between average root diameter per cutting and the number of roots measured **(Figure 6)**. The regression analysis showed that 66 % of the variance in average root diameter is explained by the number of roots. The mean root diameter of the single seedling root was significantly larger than the mean root diameter of the cutting roots irrespective of hedge age **(Figure 5)**.



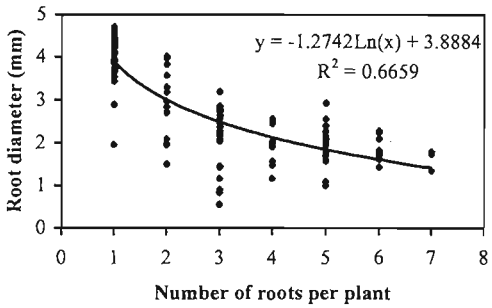


Figure 5. The average root diameter per plant at the point of emergence measured in Trial 3. Treatment means significantly different from one another are indicated by a different letter ($p<0.05$).

Figure 6. A logarithmic regression between the number of roots per plant and average root diameter in Trial 3.

Root dry mass

In addition to the declining root number, root dry mass (RDM) assessed after the 45-day bag study further indicated a decline in the root system quality with hedge age, which was significant in Trial 3 **(Figures 7 and 8)**.

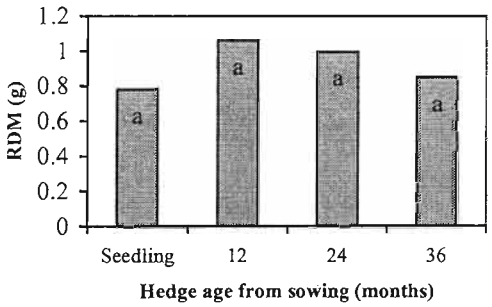


Figure 7. Average root dry mass after 45-day bag study conducted in Trial 1. Treatment means significantly different from one another are indicated by a different letter ($p<0.05$).

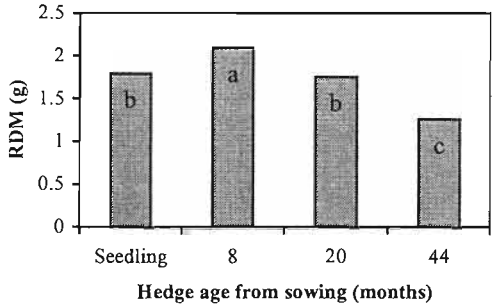


Figure 8. Average root dry mass after 45-day bag study conducted in Trial 3. Treatment means significantly different from one another are indicated by a different letter ($p<0.05$).

From this data, it would appear that root system quality, as defined by root number and dry mass, declines more rapidly than a decline in rooting efficiency. This phenomenon has also reported in *Larix* cuttings (Peer and Greenwood, 2001), suggesting that, if cuttings from older hedges possessing poorer root systems grow slower after planting, the maturation state of hedges may not always be correctly determined by using exclusively rooting efficiency. A study, conducted on *Pseudotsuga menziesii* cuttings (Ritchie *et al.*, 1993), showed that a poor quality root system impacted negatively on field performance. It could be assumed, therefore, that the significant decline in root system quality observed in these trials at such an early age may carry over into the field, negating some of the improvements expected due to superior genetic constitution.

Although some studies have shown that cutting quality from older hedges can be significantly improved by manipulating nursery procedures such as implementing bottom heat and leaving cuttings to root for longer periods in the rooting environment (Brix, 1973; Russell and Grossnickle, 1989), maturation effects may exert a greater influence and nursery improvements will only partly resolve the issue. A study conducted on the nursery and field performance of *Pinus taeda* cuttings showed that despite similar rooting was achieved from 1 and 5-year-old tree donors and the cuttings had visibly similar root systems, the cuttings from the 5-year-old donors grew significantly worse in the field after planting (Foster *et al.*, 1987).

Stem quality

Stem size

Similar to the observations made in root system quality, a significant decline in stem dimension was also observed with increasing hedge age **(Figures 9 – 12)**. Seedling dimensions in both trials tended to be smaller than cutting dimensions. This may be explained by the fact that when cuttings are set their dimension can already be measured.

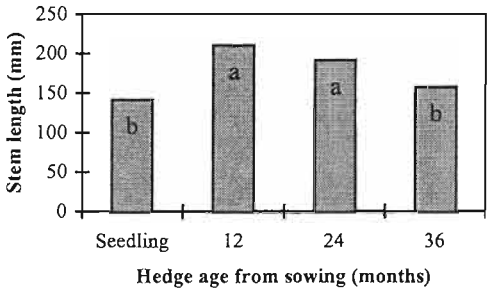


Figure 9. Stem length measured in Trial 1 at planting. Treatment means significantly different from one another are indicated by a different letter ($p<0.05$).

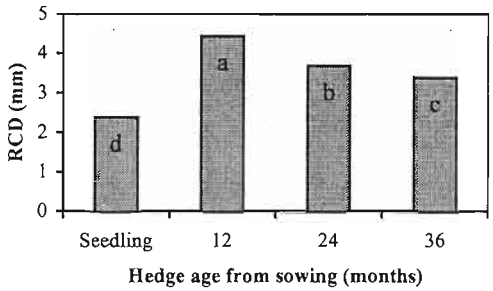


Figure 10. Root collar diameter (RCD) measured in Trial 1 at planting. Treatment means significantly different from one another are indicated by a different letter ($p<0.05$).

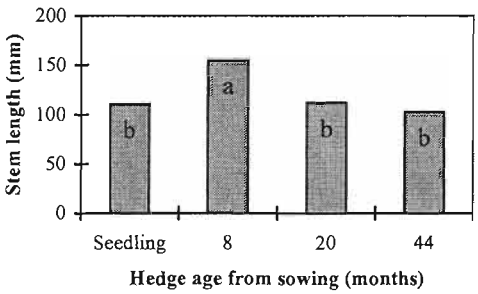


Figure 11. Stem length measured in Trial 3 at planting. Treatment means significantly different from one another are indicated by a different letter ($p<0.05$).

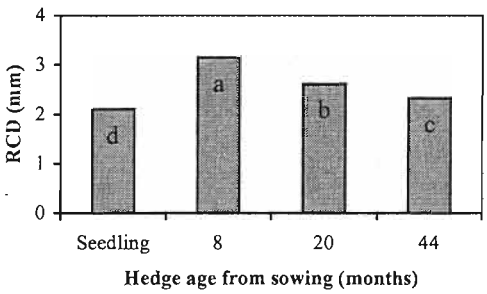


Figure 12. Root collar diameter (RCD) measured in Trial 3 at planting. Treatment means significantly different from one another are indicated by a different letter ($p<0.05$).

Improving plant dimensions of cuttings collected from older hedges may be possible through harvesting shoots more selectively. The relationships between root collar diameter and root number or root dry mass obtained in Trial 3, indicate that the root collar diameter at planting has a significant ($p<0.01$) influence on root architecture as seen by an increase in the number of roots **(Figure 13)** and root dry mass **(Figure 14)**. Furthermore, the relationship between root dry mass after the nursery bag study, and plant biomass index at planting, reveal a significant ($p<0.01$) allometric relationship **(Figure 15)**. From this it could be postulated that as hedges mature, it may become increasingly important to harvest shoots with a thicker stem, which in-turn would translate into better root and shoot growth.

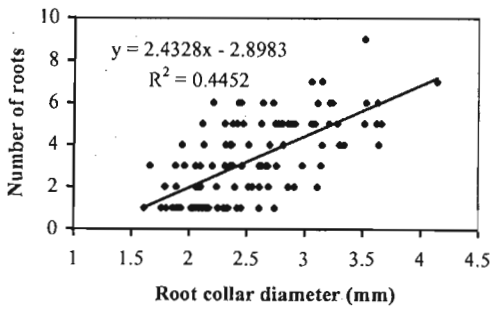


Figure 13: Linear regression between the root collar diameter of cuttings at planting and their root numbers 45 days after planting in Trial 3.

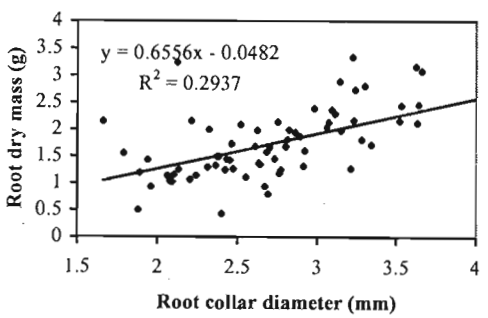


Figure 14: Linear regression between the root collar diameter of cuttings at planting and their root mass 45 days after planting in Trial 3.

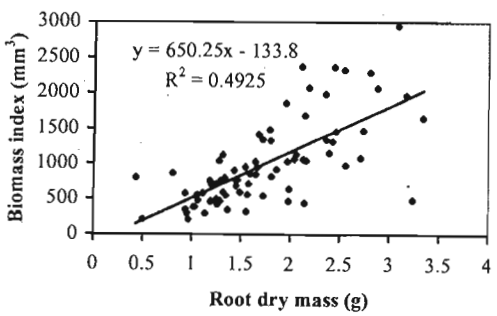


Figure 15: Linear regression between root dry mass and plant biomass index at planting in Trial 3.

Interpretation of the shoot and root measurements from different age hedges on the effects of nursery management suggests that hedge age will impact significantly. In order to dispatch even sized plants, hedges will require separation according to age categories during the harvesting and setting process to accommodate different raising periods. Neglecting to do so will result in the need for additional sorting processes during production in order to dispatch even-sized plants. If this is not done, establishment problems could be encountered when planting cuttings of different sizes. In addition, the planning required to meet demand placed by foresters on the nursery for future orders, will further have to take into account the different growth periods as there may be as much as a 2-month difference in the amount of time required to raise suitable sized cuttings from the youngest to oldest hedges.

Stem form

The stem form study revealed that plagiotropic growth habits were highly prevalent in cuttings harvested from older hedges **(Figure 16)**. Seventy percent of all cuttings harvested from the 44-month-old hedges produced cuttings that were categorised as having a poor or very poor stem form. In comparison, in excess of 95% of cuttings harvested from 8-month-old hedges, were categorised as having a good or an excellent stem form. The statistical analysis conducted on the transformed scores assigned to each plant indicated that cuttings from hedges age 20-months and older, differed significantly in their appearance ($p<0.05$) from seedlings or cuttings from 8-month-old hedges. Changes from plagiotropic to orthotropic growth patterns have been reported in *P. menziesii* (Ritchie *et al.*, 1994) and *P. taeda* (Frampton *et al.*, 2000) either during the nursery raising period or soon after field establishment. This indicates that the poor stem form observed in cuttings from older hedges in these studies, may disappear after planting.

Although needle characteristics were not assessed, it was evident during the scoring process that cuttings classed as having a poor or very poor stem form, possessed an

increase in the proportion of longer secondary needles and short primary needles. This can be clearly seen in **Figure 1 A - D**.

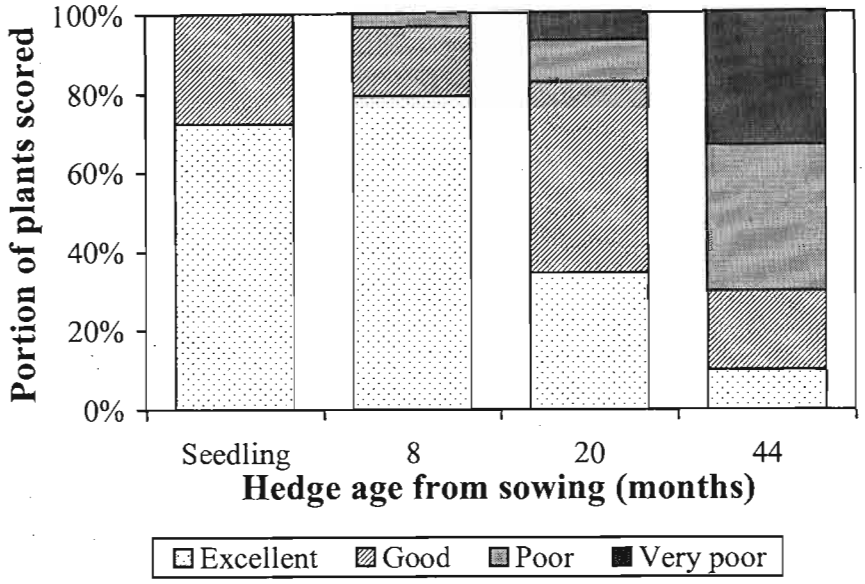


Figure 16. The proportion of stem form classes for ages assessed in Trial 3.

CONCLUSION

It was apparent that the effects of hedge maturation are manifest in cuttings harvested from seedling hedges younger than 3 years from the date of sowing. This was evidenced by a decline in rooting efficiency, in shoot size at planting and in root mass and development. Furthermore, the quality of the root system, determined by a decrease in root mass and the number of roots produced, decline before rooting efficiency deteriorates. In one trial a decline in these parameters was already apparent in cuttings from 20-month-old hedges. Root collar diameter was also found to influence the number and mass of roots produced, which in-turn significantly influenced shoot growth. A practical method of improving cutting quality as hedges age, may be to harvest shoots with greater shoot diameters.

On the basis of the nursery data generated from these three trials, it would appear that hedges should not be kept for a period longer than two years from the date of sowing to ensure the production of suitable sized cuttings with a well-formed root system. Studying the field performance of cuttings from hedges of various ages in comparison to seedlings of the same genetic origin will either confirm or reject this finding. If this predisposition to poorer performance from cuttings derived from older hedges continues to be observed in the field, then the early maturation of *P. patula* will severely impact on the use of cuttings as a means of deployment. Techniques to improve on the number of cuttings produced from juvenile hedges, without causing increased physiological stress on the hedge plant, may have to be explored to improve on the feasibility of *P. patula* cutting production.

The effects of ontogenetic maturation will influence nursery policies and decision making in a significant way whether the window of opportunity to produce *P. patula* cuttings remains two years from date of sowing or more. Raising periods will vary with hedge age and planning and coordinating setting times to meet orders will have to accommodate this principle. Cuttings, harvested from hedges of differing ages, will furthermore have to be raised separately in the nursery to prevent unnecessary sorting and handling of plant material in order to achieve plants of an even size at dispatch.

ACKNOWLEDGEMENTS

The authors would like to acknowledge T. Zulu, G. Malapane, R. Tibela and J. Phonela for their assistance in data collection and capturing. Mr. C. Young is acknowledged for peer reviewing this article before submission, and Mr. J. Crous is acknowledged for his assistance with some of the statistical analysis.

Summary

The future mass propagation of elite families of *Pinus patula* by cuttings is a realistic method of deployment if the short-term performance of cuttings and seedlings are confirmed at harvesting. This will impact significantly on the future outlook of forestry in South Africa as softwood yields are improved substantially through the introduction of material of high genetic value in commercial plantings. This, however, will require significant changes in future silviculture and other management practices as foresters and plantation staff learn to regenerate, maintain, and schedule the harvesting of cutting stands according to a different set of demands as a result of the change in plant type.

Contrary to operational experience, cutting survival was similar to seedling survival in all field studies. This indicates that factors other than those that were studied and reported on, such as planting techniques, may be contributing to mortality. Also, due to the different root structure of cuttings they may be more fragile. The similar survival observed in these trials, therefore, may have been due to the close supervision given to the planting operations by the research staff. Although survival was similar, both plant types survived unacceptably poorly in the majority of studies with an average stocking of approximately 50% at one year. It is therefore anticipated that commercial stands will require several blanking operations in order to achieve an acceptable stocking in excess of 85% by the following planting season. The reduction in expected profitability as a result of blanking costs, delayed establishment, and the loss of improved genetic plant material, indicates that this is an area that still requires further research irrespective of what plant type is being planted.

The pathogen, *Fusarium circinatum*, was commonly isolated from the planting stock before and after planting in two studies. Due to its virulent nature, it was assumed that mortality on the trees on which *F. circinatum* was isolated was principally due to this pathogen. At planting all plants were observed to be healthy and free of disease indicating that this pathogen maybe carried from the nursery to the field in a cryptic form, either inside or outside the plant tissue, which results in the death of the newly planted

tree. In two field studies, where *F. circinatum* was commonly isolated, the application of Benomyl fungicide and to some extent the biological control agent *Trichoderma harzianum* at planting appeared to improve survival although this improvement was not significant. Laboratory studies, designed to determine alternatives to Benomyl fungicide, indicated that three fungicides (Octave, Folicur and Tilt), three sterilants (Sporekill®, Prasin® and Citex®), as well as a biological control agent (*T. harzianum*), were all highly successful in controlling *F. circinatum* colony growth *in vitro*. It is recommended that these products undergo nursery testing, where the plant material is inoculated with *F. circinatum* spores, in order to test their efficacy and possible phytotoxicity *in vivo* before commercial application.

Post-planting survival was also affected by site climate. Greater temperature extremes, as well as lower humidity and less rainfall resulted in poor survival. Plant dimension at planting was found to interact with site quality where it was a significant factor on a poor quality site. Optimal cutting dimensions at planting was a root collar diameter of 2.8 – 3.2 mm, and a stem height greater than 7 cm at planting for cuttings produced in cavities 90 ml in volume. Optimal seedling dimensions at planting were a root collar diameter of 1.8 – 2 mm, and a stem height of 10 – 15 cm for seedlings produced in cavities 80 ml in volume.

In a separate study, plant morphological criteria influenced medium-term growth, where greater root mass and thicker cutting root collar diameters at planting improved field growth performance for seven years after planting. A greater root mass at planting was achieved by raising cuttings in containers that could support greater medium volume. From the study it was concluded that cuttings should be raised for an approximate period of 9 months in container cavities no smaller than 80 ml in volume and possess an oven-dry root mass of 0.3 – 0.5 g at planting. In addition to similar survival, the cuttings in this study grew either similarly to, or in some cases out-performed, the seedlings that were used as a control.

Several other published studies indicate that hedge maturation poses the greatest threat to the success of softwood cutting deployment. This is especially true in clonal forestry and methods to maintain juvenility, such as cold storage of shoots and cryopreservation, require further research before clonal plantations of *P. patula* can be realised. In the studies carried out on family hedges in this report, the effect of donor hedge maturation was found to influence nursery management practice and the characteristics of rooted cuttings. The nursery data indicates that rooting efficiency, root system quality, and stem size and form, all decline with increasing hedge age particularly from two years after the date of sowing. A decline in root system quality was particularly apparent and was observed prior to a decline in rooting efficiency. If field trials indicate poorer performance from older hedges, it may be necessary to determine whether the causes are purely ontogenetic, morphological, or both before drawing final conclusions about hedge longevity. Until such results are known, it is recommended that *P. patula* cuttings should be propagated from seedling donors maintained as hedges, approximately 15 cm high, for a period not more than three years from the date of sowing.

References

ADES, P. K. and SIMPSON, J. A., 1990. Clonal selection for resistance to *Dothistroma* needle blight in *Pinus radiata*. Kluwer Adademic Publishers. New Forests 4: 27-35.

AITKEN-CHRISTIE, J. and SINGH, A. P., 1987. Cold storage of tissue cultures. **In**: BONGA, J. M and DURZAN, D. J. (eds.). Cell and Tissue Culture in Forestry, Vol. 2.

ALLAN, J. and O' DONNEL, M., 2002. Phase change and rejuvenation. Internet site: www.forestry.gov.uk/website/Oldsite.nsf/ByUnique/GGAE-548KB2.

ALLAN, J., 2003. The technology of clonal forestry of conifers. **In:** SAMUEL, S., LEE, S. and ALLAN, J. (eds). Clonal Forestry - Who Are You Kidding? Nordic Group for the Management of Genetic Resource of Trees, Scotland, 3rd to 5th September 2002.

ALLAN, R., and HIGGS, G., 2000. Methods of improving the survival of *Pinus patula* planted into harvesting residues. Southern African Forestry Journal 189: 47-51.

ALLAN, R., MORRIS, A. and CARLSON, C., 2000. Survival and early growth effects of some re-establishment practices with *Pinus patula*. Southern African Forestry Journal 187: 29-36.

ANONYMOUS, 1977. The wax coating on radiata pine needles. **In:** "What's New in Forest Research" No 52, p. 4. Forest Research Institute, Rotorua, New Zealand.

ATKINSON, P. R., 1997. Recommendations, practices and notes on the control of pests and diseases in commercial forestry in South Africa. ICFR Bulletin 8/97.

AVENTIS CROPSCIENCE, 2000 (a). Material safety data sheet on Octave. Document no. 0898 2.00 GB, Pretoria, South Africa.

AVENTIS CROPSCIENCE, 2000 (b). Material safety data sheet on Previcure N. Document no. CP 604 2.00 GB, Pretoria, South Africa.

BACON, C. W., YATES, I. E., HINTON, D. M. and FILMORE, M., 2001. Biological control of *Fusarium moniliforme* in maize. Environmental Health Perspectives, 109 (2): 325-332.

BARNES, R. D. and MULLIN, L. J., 1974. Flowering, productivity and phenology in clonal seed orchards of *P. patula*, *P. elliottii*, *P. teada* and *P. kesiya* in Rhodesia. Forest Research Paper No. 3. Rhodesia Forestry Commission, Salisbury, p 81.

BARNETT, J, P. and BRISSETTE, J. C., 1986. Producing Southern Pine Seedlings in Containers. USDA General Technical Report SO-59.

BARNETT, J. P., BRISSETTE, J. C., KAIS, A. G. and JONES, J. P., 1988. Improving field performance of southern pine seedlings by treating with fungicides before storage. Southern Journal of Applied Forestry, 12: 281-285.

BARROWS-BROADDUS, J., TEESLINK, S. D. and KERR, T. J., 1983. Characterization of *Arthrobacter* strains isolated from the pine environment that inhibit *Fusarium moniliforme* var. *subglutinans*, the causal agent of pitch canker. Canadian Journal of Microbiology, 29: 1382-1389.

BAYER, 2000. Material safety data sheet on Folicur EW 250. Document no. 346830/06. Last updated 26/04/2000. Dormagen, Germany.

BAYLEY, A. and BLAKEWAY, F., 2002. Deployment strategies to maximise value recovery from tree improvement: The experience of two South African companies. South African Forestry Journal, 195:11-22.

BAYLEY, A., 1995. The effects of seedling age, seedling quality, and post-planting field conditions on the survival of *P. patula* seedlings produced under two nursery growing regimes. Sappi Forest Research, Propagation Programme Research Report PP2/95.

BAYLEY, A., 1998. The effect of seedling age and copper root pruning on the post-planting survival, growth and root form of *P. patula* seedlings. Sappi Forest Research, Propagation Programme Research Report PP6/98.

BELL, D. K., WELLS, H. D. and MARKHAM, C. R., 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology, 72: 379-382.

BENTZER, B. G., 1988. Rooting and early shoot characteristics of *Picea abies*. (L) Karst. Cuttings originating from shoots with enforced vertical growth. Scandinavian Journal of Forestry Science, 3: 481-491.

BESTER, C., VAN DER MERWE, L. H. C. and MALEMA, J. L., 2000. Controlled pollination in *Pinus patula*: Constraints and possible solutions. South African Forestry Journal, 189: 109-117.

BHELLA, H. S. and ROBERTS, A. N., 1975. Seasonal changes in origin and rate of root initials in Douglas-fir stem cuttings. American Society for Horticultural Science, 100: 643-646.

BLACK, D. K., 1972. The influence of shoot origin on the rooting of Douglas-fir stem cuttings. Combined Proceedings International Plant Propagators' Society, 22: 142-157.

BLAKESLEY, D., WESTON, G. D. and ELLIOTT, M. C., 1992. Increased rooting and survival of *Cotinus coggygria* cuttings from etiolated stockplants. Journal of Horticultural Science, 67: 33-37.

BOLSTAD, P. V. and LIBBY, W. J., 1982. Comparisons of radiata pine cuttings of hedge and tree-form origin after seven growing seasons. Silvae Genetica, 31: 9-13.

BONGA, J. M. and VON ADERKAS, P., 1993. Rejuvenation of tissues from mature conifers and its implication for propagation *in vitro*. **In:** AHUJA, M. R. and LIBBY, W. J. (eds). Clonal Forestry 1. Genetics and Biotechnology, p 182-199.

BORCHERT, R., 1976. Differences in shoot growth patterns between juvenile and adult trees and their interpretation based on systems analysis of trees. Acta Horticulturae (ISHS), 56: 123-130.

BOWER, R. and VAN BUIJTENEN, J. P., 1977. A comparison of rooting success of greenhouse-grown and field-grown slash pine cuttings. Canadian Journal of Forest Research, 7: 183-185.

BREDENKAMP, B., 1993. Top height: A definition for use in South Africa. Southern African Forestry Journal, 167: 55.

BREDENKAMP, B., 2000. Volume and mass of logs and standing trees p. 167 - 174. **In:** OWEN, D. L. (Ed) South African Forestry Handbook: Vol. 1. Southern African Institute of Forestry.

BRIX, H., 1973. Rooting of cuttings from mature Douglas-fir. New Zealand Journal of Forest Science, 4: 133-139.

BROWNE, R. D., DAVIDSON, C. G. and GOBIN, S. M., 1996. Effects of crown position and plant age on rooting of Jack Pine long shoot cuttings. Tree Planters' Notes, 47: 100-104.

BRUTON, T. 2003. Part three: Specimen preparation for the SEM **In:** Scanning Electron Microscopy, a practical handbook for postgraduate students p 23-25. Centre for Electron Microscopy, University of KwaZulu Natal, Pietermaritzburg.

BUCKMAN LABORATORIES, 2000. Material safety data sheet on Bulab 6044. Revision No: MSDS – 03. 16/08/2000. Hammarsdale, South Africa.

BURDON, R. D. and BANNISTER, M. H., 1985. Growth and morphology of seedlings and juvenile cuttings in six populations of *P. radiata*. New Zealand Journal of Forestry Science, 15: 123-34.

BURDON, R. D. and LOW, C. B., 1991. Wood density in Seedling and Clonal *Pinus radiata*. **In:** MILLER, J. T. (ed). Clonal Forestry Workshop. p 130-131. 1-2 May 1989, Rotorua: Ministry of Forestry (New Zealand), FRI Bulletin No. 160.

BUTTERFIELD, B. G., and MEYLAN, B. A., 1980. The structure of softwoods p 28-47. **In:** Three-dimensional structure of wood, an ultrastructural approach. 2nd edition.

CAMERON, J. N., WHITEMAN, P. H. and DAVIS, J. H., 1987. Benefits of large scale production of cuttings from advanced full-sib families. Paper presented to meeting of Austrailian Research Working Group on Plantation Silviculture, Gympie, Queensland.

CAMERON, R. J. and THOMPSON, G. V., 1969. The vegetative propagation of *Pinus radiata*: Root initiation in cuttings. Botanical Gazette, 130: 242-251.

CAMERON, R. J., 1968. Propagation of *Pinus radiata* by cuttings. Influences Affecting the Rooting of Cuttings. F.R.I. Production. Forest Report No. 65.

CHAPERON, H., 1979. Maturation et bouturage des arbres forestiers. **In:** Micropropagation d'Arbres forestiers. Assoc. Foret-Cellulose, Damaine de 1/Etancon, 7730 Nangis, France. p. 19-31. (Cited by OSBORNE, R., 1992).

CITREX, 2000. Material safety data sheet on Citrex. Revised and issued on 01/05/2000. Saint Celoni (Barcelona), Spain.

CLARKE, F. B. and SLEE, M. U., 1991. Clonal management and testing in Pinebank. **In:** MILLER, J. T. (ed.). Clonal Forestry Workshop. p. 63-66. 1-2 May 1989, Rotorua: Ministry of Forestry (New Zealand), FRI Bulletin No. 160.

CLARKE, F. B., 1992. Clonal Forestry: Australia leads in commercial application of clonal forestry. Australian Forest Grower, summer, 1992, p. 10-11.

COPES, D. L., 1992. Effects of long-term pruning, meristem origin, and branch order on the rooting of Douglas-fir stem cuttings. Canadian Journal of Forest Research, 22:1888-1894.

COUTINHO, T. A., 2003. Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Personal communications.

CROUS, J. W, 2004. Post establishment survival of *Pinus patula* in Mpumalanga, South Africa, with special focus on Pitch Canker disease. Sappi Forests Research Document 25/2004. Shaw Research Centre, South Africa.

DAVIES, H. E. and AITKEN-CHRISTIE, J., 1991. Cold-storage technology update. **In:** MILLER, J. T. (ed.). Clonal Forestry Workshop. p. 74-77. 1-2 May 1989, Rotorua: Ministry of Forestry (New Zealand), FRI Bulletin No. 160.

De ASSIS, T. F., 2001. Evolution of technology for cloning eucalyptus in large scale. **In:** Proceedings: Developing the Eucalypt of the Future. IUFRO Unit 2.08.03 Improvement and culture of the Eucalypts, Valdivia, Chile 10-15 September 2001 p. 22.

De JAGER, B., 2000. Comparison of growth of *Pinus patula* seedlings and cuttings: 3 year results. **In:** Proceedings, Forest Genetics for the Next Millennium, IUFRO Working Party 2.08.01, Durban, South Africa, 8 to 13 October 2000.

DEKKER-ROBERTSON, D. L. and KLEINSCHMIDT, J., 1991. Serial propagation in Norway spruce (*Picea abies* (L.) Karst.): Results from later propagation cycles. Silvae Genetica, 40: 202-214.

DONALD, D. G. M., 1987. Vegetative propagation of pines using cuttings. South African Forestry Journal, 140: 1-35.

DOOLEY, B. J., 1986. Factors affecting rooting of conifer cuttings. Proceedings of seminar no.1. Department of Horticulture, University of Natal, Pietermaritzburg.

DOVEY, S., 2005, Institute for Commercial Forestry Research, Private Bag X01, Scottsville, 3209, South Africa. Personnel communications.

DURZAN, D. J., 1976. Biochemical changes during gymnosperm development. Acta Horticulturae (ISHS), 56: 183-194.

DVORAK, W. S., GUTIERREZ, E. A., HODGE, G. R., ROMERO, J. L., STOCK, J. and RIVAS, O., 2000 (b). *Pinus caribaea var. hondurensis*. **In:** Conservation and Testing of Tropical and Subtropical Forest Tree Species by the CAMCORE Cooperative, college of Natural Resources, NCSU, Raleigh, NC. USA. p.12-33.

DVORAK, W. S., GUTIERREZ, E. A., OSORIO, L. F., VAN DER MERWE, L., KIKUTI, P. and DONAHUE, J. K., 2000 (c). *Pinus chiapensis*. **In:** Conservation and Testing of Tropical and Subtropical Forest Tree Species by the CAMCORE Cooperative, college of Natural Resources, NCSU, Raleigh, NC. USA. p. 35-52.

DVORAK, W. S., JORDAN, A. P., PEREZ DE LA ROZA, J. and HODGE, G. R., 2000 (a). *Pinus jaliscana*. **In:** Conservation and Testing of Tropical and Subtropical Forest Tree Species by the CAMCORE Cooperative, college of Natural Resources, NCSU, Raleigh, NC. USA. p. 87-94.

DVORAK, W. S., KIETZKA, J. E., DONAHUE, J. K., HODGE, G. R. and STANGER, T. K., 2000 (d). *Pinus greggii*. **In:** Conservation and Testing of Tropical and Subtropical Forest Tree Species by the CAMCORE Cooperative, college of Natural Resources, NCSU, Raleigh, NC. USA. p. 53-72.

DVORAK, W. S., KIETZKA, J. E., STANGER, T. K. and MAPULA, M., 2000 (e). *Pinus herrerae*. **In:** Conservation and Testing of Tropical and Subtropical Forest Tree Species by the CAMCORE Cooperative, college of Natural Resources, NCSU, Raleigh, NC. USA. p. 74-86.

DVORAK, W. S., STANGER, T. K. and ROMERO, J. L., 2000 (f). *Pinus maximartinezii*. **In:** Conservation and Testing of Tropical and Subtropical Forest Tree Species by the CAMCORE Cooperative, college of Natural Resources, NCSU, Raleigh, NC. USA. p. 96-105.

DVORAK, W. S., STANGER, T. K. and ROMERO, J. L., 2000 (g). *Pinus tecunumanii.* **In:** Conservation and Testing of Tropical and Subtropical Forest Tree Species by the CAMCORE Cooperative, college of Natural Resources, NCSU, Raleigh, NC. USA. p. 189-209.

EASLEY, D. F. and LAMBETH, C. C., 1989. Sprouting and rooting potential of *Pinus oocarpa* and *P. tecunumanii* provenances. Surfit Carton de Colombia Research Report No. 125. Cali, Colombia. p. 8.

FAULDS, T. and DIBLEY, M. J., 1989. New Zealand Forest Research Institute article No.176.

FIELDING, J. M., 1954. Methods of raising Monterey pine from cuttings in the open nursery. Australian Forest Bulletin 32.

FIELDING, J. M., 1969. Factors affecting the rooting and growth of *P. r*adiata cuttings in the open nursery. Forest and Timber Bureau, Australia, Bulletin 45.

FIELDING, J. M., 1970. Trees grown from cuttings compared to trees grown from seed (*Pinus radiata* D. Don). Silvae Genetica, 19: 54-63.

FOREST STEWARDSHIP COUNCIL, 2002. Chemical pesticides in certified forests: interpretation of the FSC principals and criteria. Forest Stewardship council, Policy and Standards Unit, document no. FSC-IP-0001, Oaxaco, Mexico.

FOSTER, G. S., 1984. Heritability gains and C effects of rooting Western hemlock cuttings. Canadian Journal of Forest Research. 14: 628-638.

FOSTER, G. S., 1988. Growth and morphology of rooted cuttings and seedlings of loblolly pine and their genetic analysis. Proceedings of the 10th North American Forest Biology Workshop. p. 20-22.

FOSTER, G. S., LAMBETH, C. C. and GREENWOOD, M. S., 1987. Growth of Loblolly pine cuttings compared with seedlings. Canadian Journal of Forest Research, 17: 157-164.

FOURET, Y., ARNAUD, Y. and LARRIEU, C., 1985. In vitro rejuvenation of *Sequoia sempervirens*: Effect of number and frequency of subcultures. Research of precocious juvenility criteria. Ann. Rech. Sylv. 1984: 111-137.

FRAMPTON, J. F., LI, B. and GOLDFARB, B., 2000. Early field growth of Loblolly pine rooted cuttings and seedlings. Southern Journal of Applied Forestry, Vol. 24, No. 2.

FRAMPTON, L. J. (Jr) and HODGES, J. F., 1989. Nursery rooting of cuttings from seedlings of Slash and Loblolly pine. Southern Applied Forestry Journal, 13: 127-132.

GARCIA, J. and KUMMEROW, J., 1970. Infection of Monterey pine gratings with *Dothistroma pini*. Plant disease Reporter, 54: 403-404.

GENSTAT 5, version 3.2®., 1995. Lawes Agricultural Trust, Rothamsted Experimental Station, UK.

GIERTYCH, M., 1976. Sexual expression in pines in relation to their ontogenetic development. Acta Horticulturae (ISHS), 56: 99-104.

GIROUARD, R. M., 1973. Propagation of Spruce by stem cuttings. New Zealand Forest Journal of Science, 4: 140-149.

GOLDFARB, B., WIER, R., SURLES, S., MURTHY, R., LANZ-GARCIA, C., MASRI, R., BUSOV, V., COONEY, B., HATCHER, A. and VELEZ, E., 1997. North

Carolina State University Loblolly and Slash Pine rooted cutting program. Progress report for 1997: 4-5.

GORDON, T. R., WOOD, D. L. and STORER, A. J., 2000. Summary of the current research on pitch canker at the University of California. Pitch Canker Task Force (http://frap.cdf.ca.gov/pitch_canker/index.htm).

GREENWOOD, M. S. and NUSSBAUM, E. S., 1981. Rooting ability and vegetative growth performance of stem cuttings from one and five-year-old ortets of Loblolly pine. Proceedings of the 16th Southern Forest Tree Improvement conference. May 27-28. Blacksburg.

GREENWOOD, M. S., 1984. Phase change in Loblolly pine: shoot development as a function of age. Physiologia Plantarum, 61: 518-522.

GRENE, S., 1978. Root deformations reduce root growth and stability. **In:** VAN EERDEN, E. and KINGHORN, J. M. (Eds). Proceedings of the Symposium on Root Form of Planted Trees, Victoria, B. C. Canada, May 16 - 19, 1978. p. 150-155.

GROSSNICKLE, S.C. and FOLK, R. S., 1993, Stock quality assessment: Forecasting survival or performance on a reforestation site. Tree Planter's Notes, 44: 113-121.

HACKETT, W. P., 1985. Juvenility, maturation, and rejuvenation in woody plants, Horticultural Reviews, 7: 109-155. (Cited by HAMANN, A., 1995).

HAEMANN, J. C. and OWENS, J. N., 1972. Callus formation and root initiation in stem cuttings of Douglas fir. Canadian Journal of Forest Research, 2: 121-134.

HAFFNER, V., ENJALRIC, F., LARDET, L. and CARRON, M. P., 1991. Maturation of woody plants: a review of metabolic and genomic aspects. Annales des Sciences Forestieres (Paris), 48: 615-630.

HAINES, R. J. and NIKLES, D. G., 1987. Mass propagation of superior families and individual genotypes of some conifers in Queensland. Australian Forestry, 50: 106-111.

HAINES, R. J., WALKER, S. W. and COLEY, T. R., 1992. Morphology and rooting of shoots developing in response to decapitation and pruning of Caribbean pine. New Forests Journal, 7: 133-141.

HALLGREN, S. W. and TAUER, C. G., 1989. Root growth potential, first year survival, and growth of Shortleaf pine seedlings show effects of lift date, storage, and family. Southern Journal of Applied Forestry, 13: 163-169.

HAMANN, A., 1995. Effects of hedging on maturation in loblolly pine: rooting capacity and root formation. A thesis submitted in partial fulfillment of the requirements for the Master of Science Degree. State University of New York, college of Environmental Science and Forestry, Syracuse, New York.

HANNERZ, M., 2003. Cuttings versus seedlings - not only a matter of genetics. **In:** SAMUEL, S., LEE, S. and ALLAN, J. (eds). Clonal Forestry - Who Are You Kidding? Nordic Group for the Management of Genetic Resource of Trees, Scotland, 3rd to 5th September 2002.

HUANG, L. C., LUIS, S., HUANG, B. L., MURASHIGE, T., MAHDI, E. M. and VAN GUNGY, R., 1992. Rejuvenation of *Sequoia sempervirens* by repeated grafting of shoot tips onto juvenile rootstocks *in vitro*. Plant Physiology, 98:166-173.

HYGROTECH, 2000. Material safety data sheet on Sporekill. Issued on 05/11/2000. Atlantis, South Africa.

IVORY, M. H., 1972. Resistance to *Dothistroma* needle blight induced in *Pinus radiata* by maturity and shade. Transactions of the British Mycological Society, 59:205-212.

JACOBS, M. R., 1939. A study on the effect on sway of trees. Australian Commonwealth Forest Bulletin 26: 19.

JAMES, R. L., DUMROESE, R. K., WENNY, D. L., MYERS, J. F., and GILLIGAN, C. J., 1987. Epidemiology of *Fusarium* on containerised Douglas-fir seedlings. 1. Seed and seedling infection, symptom production, and disease progression. United States Department of Agriculture, Forest Pest Management Report no. 87-13.

JAMES, R. L., GILLIGAN, C. J. and REEDY, V., 1988. Evaluation of root diseases of containerized conifer seedlings at the Champion Timberlands Nursery. USDA Forest Pest Management Report No. 88-11.

JAYAWICKRAMA, K. J. S., JETT, J. B. and McKEAND, S. E., 1991. Rootstock effects in grafted conifers: a review. New Forests, 5: 157-173. (Cited by HAMANN, A., 1995).

KANZLER, A., 2004. Genetic gain estimates for volume at 5 years for *Pinus patula* families in 9 progeny trials testing the progeny of clones present in the Sappi Clonal Seed Orchard's & Clone Bank. Sappi Forests Research Document no. 11/04, Shaw Research Centre, South Africa.

KANZLER, A., 2005. Programme Leader: Softwoods. Shaw Research Centre. Sappi Forests Research, South Africa. Personal communications.

KARLSSON, I. and RUSSELL, J. H., 1990. Comparisons of yellow cypress trees of seedling and rooted cutting origins after 9 and 11 years in the field. Canadian Journal of forest Research, 20: 37-42.

KLOMP, B. K. and HONG, S. O., 1985. Performance of *Pinus radiata* seedlings and cuttings at age 15 years. New Zealand Journal of Forestry Science, 15: 281-297.

KOLB, P. F. and ROBBERECHT, R., 1996. High temperature and drought stress effects on survival of *Pinus ponderosa* seedlings. Tree Physiology, 16: 665-672.

KUHLMAN, E. G., DIANIS, S. D. and SMITH, T. K., 1982. Epidemiology of pitch canker disease in a loblolly pine seed orchard in North Carolina. Phytopathology, 72: 1212-1216.

LAING, M. and MORRIS, M., 2001. Biological control and pro-biotica for seed and clonal production. Presentation at the Forest Plant Production Working Group Meeting held on 05/04/2001, Pietermaritzburg, South Africa.

LANDIS, T. D., 2002. The target seedling concept – a tool for better communication between nurseries and their customers. **In:** REILEY, L. E., DUMROESE, R. K. and LANDIS, T. D. (eds). National Proceedings: Forest and Conservation Nursery Association, Rocky Mountain Research Report P 28: 12 -16.

LEAKEY, R. R. B., DICK, J. and NEWTON, A. C., 1992. Stock-plant derived variation in rooting ability: the source of physical youth. Institute Forest Terrestrial Ecology, Scotland.

LEWIS, C. S., 2000. The ageing process of trees: Discover how trees age naturally and learn how to imitate this on your bonsai. Internet site: www.btinternet.com/~colinlewis.bonsai/Reading/ageing.html.

LIBBY, W. J. and CONKLE, M. T., 1966. Effects of auxin treatment, tree age, tree vigor and cold storage on rooting young Monterey pine. Forest Science, 12: 484-502.

LIBBY, W. J. and HOOD, J. V., 1976. Juvenility in hedged Radiata pine. Acta Horticulturae (ISHS), 56: 91-98.

LIBBY, W. J., BROWN, A. and FIELDING, J. M., 1972. The effects of hedging radiata pine on production, rooting and early growth of cuttings. New Zealand Journal of Forest Science, 2: 263-283.

MAHDI, E. F. M., 1985. Phase reversal in woody perennials by grafting of adult shoot apices onto juvenile rootstocks *in vitro*. Ph.D. dissertation, University of California, Riverside, California, p. 208.

MASCHKE, J. and WEISER, F., 1988. Zur autovegetativen Vermehrung der Douglasie (*Pseudotsuga menziesii* (Mirb.) Franco). Beitrage fur die Forstwirtschaft, 22: 114-121. (Cited by HAMANN, A., 1995).

Mc CUBBIN, P. D. and SMITH, I. E., 1991.The influence of four seedling tray types over time on root growth and other growth parameters of *Eucalyptus grandis* seedlings. p143 - 155 **In:** SCHONAU, A. P. G. (Ed). Proc.: Intensive forestry: The role of eucalypts. IUFRO Symposium P2.02-01 Productivity of eucalypts, Sept. 2-6, 1991, Durban, South Africa.

Mc TAGUE, J. P. and TINUS, R. W., 1996. The effects of seedling quality and forest site weather on field survival of Ponderosa pine. Tree Planter's Notes, 47: 16-23.

MENZIES, M. I. and KLOMP, B. K., 1988. Effects of parent age on growth and form of cuttings. **In**: MENZIES, M. I., AIMERS, J. P. and WHITEHOUSE, L. J. (eds.). Workshop on Growing Radiata Pine from Cuttings. p. 18-41. May 5-7, Rotorua: Ministry of Forestry (New Zealand), FRI Bulletin No. 135.

MENZIES, M. I., FAULDS, T., DIBLEY, M. and AITKEN-CRISTIE, J., 1985. Vegetative propagation of radiata pine in New Zealand. **In**: SOUTH, D. B. (ed.). Proceedings of the International Symposium on Nursery Management Practices for Southern Pines. p. 167-190.

MENZIES, M. I., KLOMP, B. K. and HOLDEN, D. G., 1991. Optimal physiological age of propagules for use in clonal forestry. **In:** MILLER, J. T. (ed.). Clonal Forestry Workshop. p. 142-145. 1-2 May 1989, Rotorua: Ministry of Forestry (New Zealand), FRI Bulletin No. 160.

MEXAL, J. G. and LANDIS, T. D., 1990. Target seedling concepts: height and diameter: USDA General Technical Report RM 200: 17-35.

MITCHELL, R. G., 2004. The effect of plant age and container type on *P. elliottii x P. caribaea* cutting performance. Trial PR0324 file note. Sappi Forests Research. Internal document.

MITCHELL, R. G. and JONES, N. B., 2005. A comparison between *Pinus patula* seedling and cutting re-establishment success with specific focus on disease control, shoot morphology and site quality. Sappi Forests Research Document No. 06/2005. Shaw Research Centre, South Africa.

MITCHELL, R. G., ZWOLINSKI, J. and JONES, N. B., 2004 a. The effects of ontogenetic maturation in *Pinus patula* – Part I: nursery performance. Southern African Forestry Journal, 202: 29-36.

MITCHELL, R. G., ZWOLINSKI, J., JONES, N. B. and BAYLEY, A. D., 2005. Root volume and raising period affect field performance of *Pinus patula* cuttings in South Africa. Southern African Forestry Journal 204: 15-21.

MITCHELL, R. G., ZWOLINSKI, J., JONES, N. B., and COUTINHO, T., 2004 b. The effect of applying prophylactic measures on the post-planting survival of *Pinus patula* in South Africa. Southern African Forestry Journal, 200: 51-58.

MORRIS, A., 1990 a. Harvesting slash, *Hylastes angustatus* and climate factors influencing *Pinus patula* transplant survival. Usutu Pulp Company Forest Research Document 9/90.

MORRIS, A., 1990 b. Report on trial series R125 mortality of *Pinus patula* transplants due to *Hylastes angustatus* attack in relation to planting date and site elevation. Forest Research Document 8/90. Usutu Pulp Co, Swaziland.

MORRIS, A., 1991. Recommendations to improve transplant survival for re-establishment in the Usutu forest. Forest Research document 3/91. Usutu Pulp Company, Swaziland.

MORRIS, A.R., 1993. Trial series R172 final report: Seedling quality and late season planting of *Pinus patula*. Forests Research Document No. 16/93. Usutu Pulp Company, Swaziland.

MORRIS, A.R., 1994. Trial series R176 final report: Factors influencing post-planting survival in late season re-establishment of *Pinus patula*. Forests Research Document No. 16/94. Usutu Pulp Company, Swaziland.

MUHAMMAD, S. and AMUSA, N. A., 2003. In-vitro inhibition of growth of some seedling blight inducing pathogens by compost-inhabiting microbes. African Journal of Biotechnology, 2: 161-164.

NASH, S. M. and SNYDER, W. C., 1962. Quantitative estimations by plate counts of propagules of the rot *Fusarium* in field soils. Phytopathology, 52: 451-454.

NEL, B. and VILJOEN, A., 2004. Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Personal communications.

NELSON, P. E., TOUSSOUN, T. A. and MARASAS, W. F. O., 1983. *Fusarium* species: An Illustrated Manual for Identification. The Pennsylvania State University Press, University Park, Pp 193.

NICHOLLS, J. W. P., PAWSEY, C. K. and BROWN, A. G., 1976. Further studies on the ortet-ramet relationship in wood characteristics of *Pinus radiata*. Silvae Genetica, 25: 73-79.

NIRENBERG, H., 1976. Untersuchungen über die morphologische und biologische differenzieriung in der *Fusarium* - sektion *Liseola*. Mitteilungen aus der Biologischen Bundesanstalt für Land-und Forstwirtschaft. Berlin-Dahlem, 169: 1-117.

OLESEN, P. O., 1978. On cyclophysis and topophysis. Silvae Genetica, 27: 173-178.

OSBORNE, R. E., 1992. Factors affecting rooting in *Pinus patula* Scheide *et* Deppe Stem Cuttings. M.Sc. Thesis. University of Natal, Pietermaritzburg, R.S.A.

OSORIO, L. F., 1991. Vegetative propagation of *Pinus maximinoi* and *Pinus tecunumanii* by rooted cuttings. M.Sc. Thesis. North Carolina State University, Raleigh, USA.

OSORIO, L. F., 1993. Propagation and field performance of *Pinus oocarpa*, *Pinus maximinoi* and *Pinus tecunumanii* rooted cuttings. Research Report No. 150, Smurfit Carton de Colombia.

OSORIO, L. F., 2000. Eight-year results of field trials of *Pinus oocarpa*, *Pinus maximinoi*, and *Pinus tecunumanii* rooted cuttings. Surfit Carton de Colombia. Research Report (in press). (Cited by DVORAK *et al.*, 2000. *Pinus oocarpa*. **In:** Conservation and Testing of Tropical and Subtropical Forest Tree Species by the CAMCORE Cooperative, college of Natural Resources, NCSU, Raleigh, NC. USA. Pp.128-147).

OXFORD UNIVERSITY PRESS, 2000. Dictionary of Biology. Website: http://www.oup.co.uk.

PALLETT, R. 1999. Frost risk and distribution across Sappi land holdings. Sappi Forests Research Document No. 06/99. Shaw Research Centre, South Africa.

PAWSEY, C. K., 1971. Development of grafts of *Pinus radiata* in relation to age of scion source. Australian Forest Research, 5: 15-18.

PEER, K. R. and GREENWOOD, M. S., 2001. Maturation, topophysis and other factors in relation to rooting in *Larix*. Tree Physiology, 21: 267-272.

PENMAN, J. T. D., 1988. Volume, taper, and bark thickness in seedlings and cuttings from Mamaku Forest, New Zealand. New Zealand Journal of Forestry Science, 18: 311-317.

POWER, A. B., DODD, R. S. and LIBBY, W. J., 1994. Effects of hedging on maturation in Radiata pine: Western Gall Rust Susceptibility. Silvae Genetica, 43: 1-7.

PRASIN AGRI, 1999. Material safety data sheet on Prasin. Issued on 28/09/1999. Chemical Process Technologies (Pty) Ltd, Pretoria, South Africa.

REINES, M., 1963. The influence of age of clonal parent on rooting of needle bundles. GA Forest Research Council paper 16: 3.

RITCHIE, G. A., DUKE, S. D. and TIMMIS, R., 1994. Maturation in Douglas-fir: II. Maturation characteristics of genetically matched Douglar-fir seedlings, rooted cuttings and tissue culture plantlets during and after 5 years of field growth. Tree Physiology, 14: 1261-1275.

RITCHIE, G. A., TANAKA, Y., MEADE, R. and DUKE, S. D., 1993. Field survival and early height growth of Douglas-fir rooted cuttings: relationship to stem diameter and root system quality. Forest Ecology and Management, 60: 237-256.

ROBERTS, A. N. and MOELLER, F., 1978. Speculations relating to the loss of rooting potential with ageing of Douglas fir stock plants. Plant Propagator, 24: 2-4.

ROLANDO, C. A. and LITTLE, K. M., 2004. Early survival and growth responses of *Pinus patula* seedlings to planting treatments and micro-environment in two pilot trials. Institute for Commercial Forestry Research, Bulletin no 06/2004.

ROSE, R., ATKINSON, M., GLEASON, J. and SABIN, T., 1991. Root volume as a grading criterion to improve field performance of Douglas-fir seedlings. New Forests, 5: 195-209.

ROSE, R., CARLSON, W. and MORGAN, P., 1990. The target seedling concept. **In:** ROSE, R., CAMBELL, S. and LANDIS, T. (eds). Target Seedling Symposium Proceedings, Western Forest Nursery association; August 13 – 17, 1990, Roseburg.

ROULUND, H., 1975. The effect of the cyclophysis and topophysis on the rooting and behaviour of Norway Spruce cuttings. Acta Horticulturae (ISHS), 54: 39-50.

RUSSELL, J. H. and GROSSNICKLE, S. C., 1989. Rooting of yellow cypress cuttings. Part 1: Influence of donor plant maturation. FRDA Research Memo No. 083.

SLEE, M. U. and CLARKE, F. B., 1991. Stages of Development of *Pinus radiata* and implications for clonal forestry. **In**: MILLER, J. T. (ed.). Clonal Forestry Workshop. p 106-109. 1-2 May 1989, Rotorua: Ministry of Forestry (New Zealand), FRI Bulletin No. 160.

SMITH, D. R., 1986. Chapter 10: Forest and Nut Trees. 1. Radiata Pine. Pp 274-292. **In:** BAJAJ, Y. P. S. (ed.). Biotechnology in Agriculture and Forestry, Vol. 1: Trees 1.

SMITH, R. D. and THORPE, T. A., 1975. Root initiation in cuttings of *Pinus radiata* L. developmental sequence. Journal of Experimental Botany 26: 184-192.

SOUTH, D. B. and MITCHELL, R. G., 2005. A root-bound index for container grown pines. **In:** COLOMBO, S. J., DANG, Q. and BETTS, L. (eds). The Thin Green

Line: A Symposium on the State of the Art in Reforestation. July 26 to 28, 2005. Vallhalla Inn, Thunder Bay, Ontario, Canada.

SOUTH, D. B., HARRIS, S. W., BARNETT, J. P., HAINDS, M. J. and GJERSTAD, D. H., 2005. Effect of container type and seedling size on survival and early height growth of *Pinus palustris* seedlings in Alabama, USA. Forest Ecology and Management 204: 385 – 398.

St. CLAIR, J. B., KLEINSCHMIDT, J. and SVOLBA, J., 1985. Juvenility and serial vegetative propagation of Norway Spruce clones (*Picea abies* Karst.). Silvae Genetica, 34: 42-48.

SWEET, G. B. and HARRIS, J. M., 1976. Wood properties of *Pinus radiata*: seed grown trees compared with grafts from different aged ortets. New Zealand Journal of Forestry Science, 6: 114-121.

SYNGENTA, 2001. Material safety data sheet on Tilt 250 EC. Last updated 12/01/2001. Britz, South Africa.

THOMPSON, B., 1985. Seedling morphological evaluation: what you can tell by looking. **In:** DURYEA, M. L. (ed.). Proceedings: Evaluating seedling quality: principals, procedures, and predictive abilities of major tests. Workshop held October 16 – 18, 1984. Forest Research Laboratory, Oregon State University, Corvallis.

TINUS, R. W., 1978 a. Root Form: What Difference Does it Make? **In:** VAN EERDEN, E. and KINGHORN, J. M. (Eds). Proceedings of the Symposium on Root Form of Planted Trees, Victoria, B. C. Canada, May 16 - 19, 1978. p. 11-15.

TINUS, R.W., 1978 b. Root system configuration is important to long tree life. **In:** VAN EERDEN, E. and KINGHORN, J. M. (Eds). Proceedings of the Symposium on Root Form of Planted Trees, Victoria, B. C. Canada, May 16 - 19, 1978. p. 58-62.

VAN DER SIJDE, H. A. and ROELOFSEN, J. W., 1986. The potential of pine hybrids in South Africa. South African Forestry Journal, 136: 5-14.

VENTER, C., 2004. Citrex. Personal communications. No 68 Reedbuck Cresent, Unit No 2 Corporate Park, Midrand, 1685, South Africa.

VIEITEZ, E. and VIEITEZ, A., 1976. Juvenility factors related to the rootability of Chestnut cuttings. Acta Horticulturae (ISHS), 56: 269-274.

VILJOEN, A., WINGFIELD, M. J. and MARASAS, W. F. O., 1994. First report of *Fusarium subglutinans* f. sp. *Pini* on pine seedlings in South Africa. Plant Disease, 78: 309-312.

VON WÜHLISCH, G., 1984. Propagation of Norway spruce cuttings free of topophysis and cyclophysis effects. Silvae Genetia, 6: 215-219.

WALKER, S. and HAINES, R. J., 1991. Interim strategies and nursery prescriptions for the large scale propagation of F1 hybrid cuttings. Cuttings Implementation Task Force - Report No. 1.

WALKER, S., 2002. Queensland Forest Research Institute. Personal communications. walkers@qfril.se2.dpi.qld.gov.au.

WELLS, L. G. and FRANICH, R. A., 1977. Morphology of epicuticular wax on primary needles of *Pinus radiata* seedlings. New Zealand Journal of Botany, 15: 525-529.

WILLIAMS, L. A., 2003. Nursery manager, Escarpment nursery. Sappi Forests Research. P. O. Box 372, Ngodwana, 1209, South Africa. Personal communications.

WILSON, P. J., 1998. The discipline of forest tree propagation. South African Forestry Journal, 183: 47-52.

WISE, F. C., 1994. Trip report: meetings and visits in the Pacific Northwest. Westvaco Intra-company publication.

WITHERS, L. A., 1984. Freeze preservation of cells. Pp. 608-620 **In:** VASIL, I. K. (ed.) Cell culture and somatic cell genetics of plants, Vol. 1. Academic Press.

YOUNG, E., JAMES, W. and HANOVER, W., 1976. Accelerating maturity in *Picea* seedlings. Acta Horticulturae (ISHS), 56: 105-114.

ZAGORY, D. and LIBBY, W. J., 1985. Maturation-related resistance of *Pinus radiata* to Western Gall Rust. The American Phytopathological Society, 75: 1443-1447.

ZAR, J., 1984. Biostatistical Analysis, 2nd edition. p 236-246. Prentice-Hall International (Publishers).

ZWOLINSKI, J. B. and BAYLEY, A. D., 2001. Research on planting stock and forest regeneration in South Africa. New Forests 22:59 – 74.

ZWOLINSKI, J. B., DONALD, D. G. M. and VAN LAAR, A., 1994. Regeneration procedures of *Pinus radiata* in the Southern Cape Province. Part IV: Characteristic of planting stock. South African Forestry Journal, 168: 1-6.