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THE VEGETATIVE PROPAGATION AND QUALITY SPECIFICATIONS OF *PINUS PATULA* HYBRID CUTTINGS

by

CRAIG FORD

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Supervisor: Prof. Paxie W Chirwa

Co-supervisor: Dr Nicky Jones

Co-supervisor: Dr Jacob Crous

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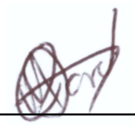
DECLARATION

I, the undersigned, hereby declare that the dissertation submitted herewith for the degree M.Sc. to the University of Pretoria, contains my own independent work and has not been submitted for any degree at any other university

Signed  ____________________________

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DEDICATION

I would like to dedicate this thesis to my wonderful family; my wife Catherine and sons Joshua and Benjamin whose love and support have meant the world to me and whose sacrifices have made this study possible.

I also dedicate this study to my parents, Lynne and Cedric Ford, whose financial support in my early studies and unwavering belief in my ability and potential, have got me to this point.

ABSTRACT

Pinus patula has for many years been the most important softwood species along the eastern regions of southern Africa but as a result of the pine pathogen, _Fusarium circinatum_, there have been serious establishment issues and difficulties in propagating _P. patula_ from either seed or cuttings. In response to this threat, research has been conducted on the development of _F. circinatum_-tolerant _P. patula_ and hybridising it with more tolerant _Pinus_ species such as _P. tecunumanii_. Not enough hybrid seed can, however, be produced to replace _P. patula_ seedling production. It has therefore become necessary to investigate the production of tolerant _P. patula_ families and various _P. patula_ hybrid alternatives through vegetative propagation. The suitability of _P. patula_ hybrids to the current and future vegetative propagation systems, that is hedges in polythene bags with composted pine bark growing media and hydroponic sand beds. It is also necessary to determine what the ideal plant specifications for those hybrid cuttings being produced are in order to ensure good survival and stocking. The potential gains from high quality planting stock include optimum stocking and volume growth. While some plant quality work has been undertaken on _P. patula_ seedlings there has not been any research into the plant quality specifications required for the successful deployment of _P. patula_ hybrid cuttings.

The objective of this study was thus to: investigate the propagation potential of these taxa in the two vegetative propagation systems, with natural infection by _Fusarium circinatum_; and to test the morphological plant quality specifications for _Pinus patula_ x _Pinus tecunumanii_ (low elevation, LE) rooted cuttings required for optimal survival and growth after planting.

The taxa propagation investigation comprised two experiments; a taxa production experiment and a propagation system experiment. Experiments were carried out at the Sappi Shaw Research Centre located near Howick, South Africa (S29°28.53' E30°10.75'). The taxa sele cted represented a range of predicted *F. circinatum* tolerance. Each experiment comprised 23 family treatments (across eight hybrid and pure taxa). A total of 2300 hedges were included in the taxa production experiment, planted in the current commercial standard which is composted pine bark growing media in black polythene bags. The propagation system experiment, compared two hedge system types, the hydroponic sand bed and current commercial standard. A total of 1200 hedges were included in this experiment. A pine mini-hedge system was employed to produce juvenile shoots for vegetative propagation in both experiments. Rooted cuttings were produced between October 2008 and June 2012. Over the 45 month period a total of 23 shoot harvests were set.

All dying hedge plants were collected and sent for laboratory confirmation of infection by *F. circinatum*. Needle samples from 493 hedges across selected hybrid crosses and *P. patula* as well as all 714 hedges of *P. patula* x *P. tecunumanii* (LE) were submitted for DNA fingerprinting to confirm their hybrid status.

Significant differences ($p < 0.001$) in mortality associated with *F. circinatum* were observed between the *P. patula* x *P. tecunumanii* (LE) hybrid (6%) and *P. patula* (19-23%). No significant differences in mortality associated with *F. circinatum* were observed within *P. patula* x *P. tecunumanii* (LE) families which ranged from zero to 15 percent. Significant mortality differences ($p < 0.001$) were observed between *P. patula* families which ranged

from eight to 44 percent. The number of rooted cuttings produced, per hedge established, over the four year period was significantly better ($p < 0.001$) in the *P. patula* x *P. tecunumanii* (LE) hybrid (52) than in *P. patula* (29-33). Significant differences ($p < 0.001$) were also observed in the number of rooted cuttings produced per family, with *P. patula* x *P. tecunumanii* (LE) families ranging from 35 to 70 cuttings per hedge plant established and *P. patula* families between 20 and 42 cuttings. Over the four year duration of the trial all taxa showed increased productivity in hedges grown in a hydroponic sand bed system, which received more consistent fertilisation and yielded an average of 55 rooted cuttings per hedge, over those grown in polythene bags with composed pine bark medium which yielded 41 cuttings on average.

To investigate the morphological plant quality requirements for *Pinus patula* x *Pinus tecunumanii* rooted cuttings, rooted cuttings aged between 2 and 23 months, from time of setting, were selected from multiple families to establish a plant quality field trial. Cuttings were grouped into five age treatments and planted in a randomised complete block design in 7 by 7 tree plots and with 6 replications. A total of 20 hybrid families were included. Cuttings were raised in 90ml inserts in a containerised system with composted pine bark growing medium. Plant quality measures were assessed across 1470 individual cuttings, with age being used as a grouping factor at field planting. The cutting quality parameters included in this study were; plant age, height, RCD, needle colour, root plug colonisation, visual presence of ectomycorrhizae and number of visible white root tips. Survival and growth for each individual cutting was recorded at one year after field planting.

The ideal raising period for *P. patula* x *P. tecunumanii* (LE) cuttings, grown in a 90ml cavity was 10 months from setting. The ideal height for cuttings was 28-32cm and the ideal root collar diameter range was 3.5mm - 4.5mm. The root plug was optimal when no growing medium fell off the plug when extracted from the insert; the root plug was firm but not hard and the plug was well colonised with a high proportion of thin brown roots. It was optimal to have at least three or more actively growing white root tips present and visible evidence of ectomycorrhizae. Needles in the dark mid-green to dark green range were shown to be optimal.

These plant quality recommendations were based on findings from a single trial site that experienced good planting conditions and good rainfall. As a result, the effects of significant water stress on the survival and growth of these cuttings was not adequately assessed and would require further testing. This study showed that the *P. patula* x *P. tecunumanii* (LE) hybrid is a feasible substitute for *P. patula* in both vegetative propagation systems, as it not only shows improved survival, through increased *F. circinatum* tolerance, but also improved productivity. It also showed that even under ideal planting conditions cutting age, height, root collar diameter, needle colour, root plug integrity and the number of white roots all had a significant effect on survival and growth of cuttings a year after field establishment.

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CHAPTER 1

INTRODUCTION

Introduction

Pinus patula Schiede ex Schlect. & Cham. *var. patula* (Mexican weeping pine) has been the single most important commercial softwood species along the eastern regions of southern Africa. By the year 2000, one million hectares of commercial *P. patula* plantations had been planted worldwide, most of which was in south and south east Africa (Dvorak, Hodge, Kietzka, et al., 2000). *Pinus patula* currently makes up 50% of all of the softwoods planted in South Africa (Mitchell *et al*., 2011).

The natural geographic range for *Pinus patula var. patula* is in the Sierra Madre Oriental from Tamaulipas to north eastern Oaxaca, Mexico (Dvorak, Hodge, Kietzka, et al., 2000), Figure 1.1a. In their natural range *P. patula* trees grow to approximately 35m in height with diameters at breast height of 0.8m, the wood is yellowish-white with moderate density and low levels of extractives (Dvorak, Hodge, Kietzka, et al., 2000). This species is primarily grown for two commercial purposes, pulpwood and saw timber. Plantations are generally grown to 18 years of age for pulpwood production and 25 years and older for saw timber processes. Species productivity in South Africa, of unimproved selections, varies between 15 m^3/ha/yr and 26 m^3/ha/yr at rotation ages of 15 to 25 years (Dvorak, Hodge, Kietzka, et al., 2000).

Unfortunately *P. patula* shows low levels of tolerance to the pine pathogen, *Fusarium circinatum* (teleomorph= *Gibberella circinata*) (Nirenberg and O'Donnell, 1998) (Viljoen *et al*. 1995, Coutinho *et al*. 2007, Wingfield *et al.* 1999, 2008, Mitchell *et al*. 2012a). Originating in Mexico (Britz et al., 2001; Wikler and Gordon, 2000) this pathogen has rapidly spread throughout the world. The pathogen was initially recorded in the south east United States of America in 1946 (Hepting and Roth, 1946) and subsequently in California in 1986 (Gordon et al. 2001), Japan in 1986

(Kobayashi and Muramoto, 1989), South Africa in 1990 (Viljoen et al., 1994), South Korea in 2000 (Lee et al., 2000), Chile in 2002 (Wingfield et al., 2002), Spain in 2005 (Landeras et al., 2005), Columbia in 2005 (Steenkamp et al., 2012), Italy in 2007 (Carlucci et al., 2007), Portugal in 2009 (Bragança et al., 2009) and in Uruguay in 2009 (Alonso and Bettucci, 2009). The effects of this pathogen on mature trees have been widely reported and they typically show pitch cankers, with severe resin flow covering the stems and branches and resin soaked wood, with smaller branches being girdled and showing the typical dieback 'flagging' symptom of the disease (Dwinell *et al*., 1985; Mitchell et al., 2011). Limited instances of this manifestation of the disease have been observed in South Africa (Coutinho et al., 2007) but only on *Pinus radiata* D. Don and only within a small geographical area in the Western Cape.

Within South Africa *Fusarium circinatum* has mostly been encountered as a nursery and establishment related disease. The nursery disease occurs on roots and base of the stem (root collar) (Viljoen et al., 1994) of seedlings in the nursery and in the months after establishment in the field. The formation of a resinous soaking of the root collar region of the seedlings, in response to the infection, causes root dieback and an inability to take up water (Mitchell et al., 2011; Wingfield et al., 2008). This results in the primary symptoms of infection which are tip wilting and then a blue/purple discoloration at the wilting point of the plant. Finally, the seedlings die and turn red-brown. Nursery losses in the country were most severe during the mid-1990's and early 2000's but improved nursery hygiene measures and reduced physiological stresses have successfully reduced the nursery losses to below 3% of the crop in the large commercial nurseries (Morris, 2010). Since the mid-2000's significant field mortality effects, at establishment, have been observed. Survival of *P. patula* in field, before this time, was approximately 75-90% while during 2004 and

2005 it had reduced to 30-40% (Morris, 2010). Crous (2005) found 42% of mortality could be positively associated with the disease during the 2004 and 2005 season. Mitchell et al. (2009) used current establishment costs for pitting, planting and blanking to calculate the cost of these losses to be in the region of R602 ha^{-1} for the saw timber industry, and R896 ha^{-1} for the pulpwood industry which translated into an annual loss of R11 million to the industry for *P. patula* alone, where approximately 15 000 ha are planted annually. *Fusarium circinatum* has thus become one of the largest threats to the pine forestry industry in South Africa having resulted in up to 50% post planting mortality (Morris, 2010). With considerable breeding resources invested in the improvement of growth and wood properties of *P. patula,* most large forestry companies in South Africa are understandably hesitant to entirely abandon the species. Two strategies for reducing the losses associated with *F. circinatum* are therefore being pursued by the industry, that is; making selections of more tolerant geographic races and families within *P. patula* and also hybridising *P. patula* with more tolerant *Pinus* species such as *P. tecunumanii* and *P. oocarpa*.

Pinus tecunumanii Eguiluz & J. P. Perry naturally occurs from the highlands of central Chiapas, Mexico to central Nicaragua (Dvorak et al., 2000b) (Figure 1.1d). This species comprises two distinct groups: (1) Those from lower elevation sources (450-1500m above sea level) in Nicaragua, Honduras and Belize and (2) those from high elevation sources (1500-2900m) in Honduras, Guatemala and Mexico (Dvorak et al., 2000b). Both the high and low elevation geographical ranges show better tolerance to *F. circinatum* than *P. patula* (Hodge and Dvorak, 2006) but the low elevation source shows higher tolerance than the high elevation sources (Hodge and Dvorak 2000). The high elevation sources do however show improved cold tolerance over the low elevation (Hodge et al. 2012). In addition to improved *F. circinatum* tolerance in *P. tecunumanii* over *P. patula*, this species also offers improved drought tolerance and higher productivity (Hodge and Dvorak, 2012).

Pinus oocarpa Schiede ex Schlechtendal *var. oocarpa*, is a common Mesoamerican pine whose geographical range extends from southern Sonora, Mexico to northern Nicaragua (Dvorak et al., 2009) (Figure 1.1c). Although *P. oocarpa* shows slightly better tolerance to *F. circinatum* than even *P. tecunumanii* (Hodge and Dvorak, 2006) it is now regarded as a less important plantation species as *P. tecunumanii* is faster-growing in the tropics and subtropics (Dvorak et al. 2000a).

In order to fully test all possibly beneficial hybrid combinations, Sappi Forests has also included *P. greggii* in their hybrid combinations. Two varieties of *Pinus greggii* exist; (1) the northern populations from Nuevo León and Coahuila, Mexico, known as *P. greggii* Engelm. Ex Parl. *var. greggii*, and (2) those in the states of Hidalgo, Puebla, Querétaro, San Luis Potosí and Veracruz, known as *P. greggii* Engelm. ex Parl. *var. australis* Donahue & López-Upton (Dvorak et al., 2000d) (Figure 1.1b). While *P. greggii* offers excellent drought and cold tolerance (Dvorak et al., 2000d) it, like *P. patula*, shows extreme susceptibility to *F. circinatum* (Hodge and Dvorak, 2006).

Testing of various *P. patula* hybrid combinations have been undertaken by various companies within South Africa with the guidance of the CAMCORE cooperative (Mitchell et al., 2012, 2013), including Sappi Forests. In order to test the *F. circinatum* tolerance predictions made for the pure species (Hodge and Dvorak, 2000) in hybrid combinations with *P. patula*, Sappi Forests embarked on multiple controlled pollinations using *P. tecunumanii* (Low and High elevation sources), *P. oocarpa* and *P. greggii* (Northern and Southern varieties) pollen.

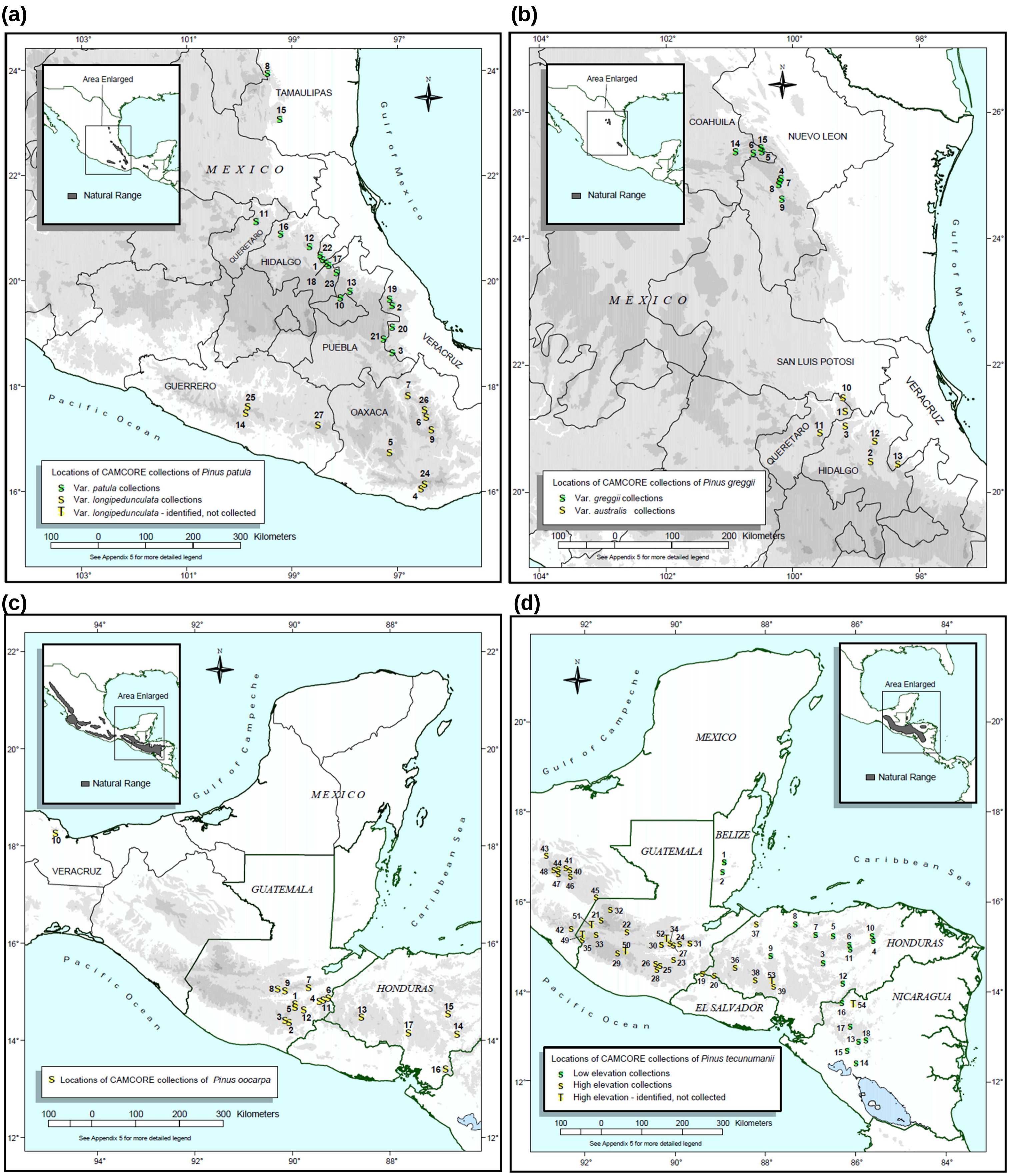

Figure 1.1: Geographic ranges of *P. patula* (a), *P. greggii* (b), *P. tecunumanii* (c) and *P. oocarpa* (d) collections completed by the CAMCORE cooperative for testing by members. (in: Dvorak et al., 2000a, 2000b, 2000c,2000d)

The production of this hybridised, control-pollinated seed was completed using cherry pickers. This time consuming, expensive and laborious task yielded low amounts of viable hybrid seed. This observation is common for this procedure and has resulted in hybrid taxa being produced elsewhere though vegetative means in order to multiply the genetic gain derived.

To test the new Sappi Forests hybrid combinations it was necessary to investigate their potential for use in a vegetative propagation system, and how to optimise this, while also producing sufficient numbers of plants for testing in field trials and laboratory inoculation trials. The basis for the vegetative propagation system used was the current commercial production hedge mother stock systems in place for the *P. elliottii* x *P. caribaea* hybrid and previously for *P. patula* (which had to be abandoned due to the mortality extremes experience as a result of *F. circinatum*). This system involves hedges established in polythene bags with composted pine bark as the growing medium. The suitability of *P. patula* hybrids to this vegetative propagation system has not been established, nor have alternative systems been investigated for the vegetative propagation of pines in South Africa, for example the hydroponic sand bed systems currently in use in Brazil (de Assis *et al*. 2004).

An important consideration in forest establishment success is the planting of high quality plants with good survival potential. For this reason nurseries need to produce plants with attributes that allow for the best chance of success after planting (Grossnickle, 2012). Since 2002, Sappi Forests has implemented quality control measures, and a system of measuring and recording these parameters, in its commercial nurseries in order to ensure the maximum survival and growth potential of seedlings and cuttings in-field. The existing specifications, developed prior to 2002, were based on quantitative trial information and on qualitative nursery experience. None of these early trials, however, were specifically designed to test all possible specifications simultaneously and most focussed on pure species seedlings. No work has previously been completed on the quality specifications for *P. patula* hybrid cuttings in Sappi or the industry at large. It has thus become necessary to determine not only if we can produce alternate tolerant hybrid crosses, but also what the ideal plant specifications for those hybrids might be to ensure good survival and growth.

1.1 Problem statement

While some work has been conducted on the vegetative propagation and successful establishment requirements of *P. patula as a* pure species, the suitability of *P. patula* hybrids to the existing vegetative propagation systems and newer hydroponic systems has not been established. While some plant quality work has been undertaken on *P. patula* seedlings there has not been any research into the plant quality specifications required for the successful deployment of *P. patula* hybrid cuttings.

1.2 Main objective

The main objective of this study was to determine the feasibility of propagating *P. patula* hybrids through rooted cuttings and to determine the required morphological specifications for those cuttings to ensure successful field establishment and growth.

1.2.1 Specific objectives and associated research questions

The following specific objectives and associated questions were developed to achieve the general objective of the study:

Specific objective 1: To test the potential of various *P. patula* hybrids for vegetative propagation in the existing vegetative propagation systems employed at the Sappi commercial pine cuttings nursery as well a new hydroponic 'sand bed' system being developed for a new cuttings nursery.

a) Can *P. patula* hybrids be propagated through vegetative means?
b) Which propagation system would be the most productive means of producing *P. patula* hybrid cuttings?
c) Do the *P. patula* hybrids, selected for tolerance to *F. circinatum*, show improved survival over the pure species in the propagation system?
d) Are there propagation system survival differences in *P. patula* and *P. patula* hybrids?
e) Is there potential to select within *P. patula* and *P. patula* hybrid families to improve survival and rooted cutting production of hedges?

Specific objective 2: To test the effect of various possible morphological plant quality measures on field establishment success, in terms of survival and growth, in *P. patula* x *P. tecunumanii* (LE) rooted cuttings.

a) Do cutting age, height, root collar diameter, leaf colour, ectomycorrhizae presence and growing white root tip prevalence have a significant effect on cutting survival in field?
b) What are the optimal ranges for those morphological parameters which showed significant effects on cutting survival?
c) Do cutting age, height, root collar diameter, leaf colour, ectomycorrhizae presence and growing white root tip prevalence have a significant effect on cutting growth in field?
d) What are the optimal ranges for those morphological parameters which showed significant effects on cutting growth in field?
e) What would the combined ideal recommendations for cutting age, height, root collar diameter, leaf colour, ectomycorrhizae presence and growing white root tip prevalence be, to maximise both growth and survival?

1.3 Justification

Considering the importance of pine, *Pinus patula* in particular, to the South African forestry industry it is essential that we are able to find a means of overcoming the severe risks imposed by the *Fusarium circinatum* pathogen on *P. patula* seedling establishment. With Sappi forests, like many other companies in South Africa, predominantly deploying highly susceptible *P. patula* seedlings as their main pine species, it is essential that alternatives are investigated and well understood and that their commercial possibilities are considered. It is thus essential that we understand how to produce these hybrids through vegetative means, and that we can confirm the tolerance we expect to see and that productivity in vegetative propagation systems is sufficiently good to be able to meet the supply demands for the hybrid.

In order to ensure the successful deployment of these cuttings, in a previously seedling dominated market, it is essential that we understand the quality requirements for successful field establishment.

1.4 Structure of the thesis

This thesis is paper based and comprises a background to the study, two articles submitted for journal publication and a concluding chapter with recommendations based on this study.

CHAPTER 2

PINUS PATULA AND PINE HYBRID HEDGE PRODUCTIVITY IN SOUTH AFRICA: A COMPARISON BETWEEN TWO VEGETATIVE PROPAGATION SYSTEMS EXPOSED TO NATURAL INFECTION *BY FUSARIUM CIRCINATUM*. SOUTHERN FORESTS (IN PRESS)

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Pinus patula* and pine hybrid hedge productivity in South Africa: a comparison between two vegetative propagation systems exposed to natural infection by *Fusarium circinatum

CM Ford*[1,2], NB Jones[1] and PWC Chirwa[2]

[1]Sappi Forests, Shaw Research Centre, PO Box 473, Howick, 3290, South Africa

[2]Forest Science Postgraduate Programme, Department of Plant Production and Soil Science, University of Pretoria, Pretoria, 0002, South Africa

** Corresponding author, email: craig.ford@sappi.com*

In response to the *Fusarium circinatum* pine pathogen threat in Southern Africa, research has been conducted on the development of *F. circinatum*-tolerant *P. patula* and *P. patula* hybrids. The objective of this study was to investigate the propagation potential of these taxa in two vegetative propagation systems, hydroponic sand beds and polythene bags with composted pine bark growing media. Significant differences ($p < 0.001$) in mortality associated with *F. circinatum* were observed between the *P. patula* x *P. tecunumanii* (LE) hybrid (6%) and *P. patula* (19-23%). No significant differences in mortality associated with *F. circinatum* were observed within *P. patula* x *P. tecunumanii* (LE) families which ranged from zero to 15 percent. Significant mortality differences ($p < 0.001$) were observed between *P. patula* families which ranged from eight to 44 percent. The number of rooted cuttings produced, per hedge established, over the four year period was significantly better ($p < 0.001$) in the *P. patula* x *P. tecunumanii* (LE) hybrid (52) than in *P.*

patula **(29-33). Significant differences ($p < 0.001$) were also observed in the number of rooted cuttings produced per family, with *P. patula* x *P. tecunumanii* (LE) families ranging from 35 to 70 cuttings per hedge plant established and *P. patula* families between 20 and 42 cuttings. Over the four year duration of the trial all taxa showed increased productivity in hedges grown in a hydroponic sand bed system, which received more consistent fertilisation and yielded an average of 55 rooted cuttings per hedge, over those grown in polythene bags with composted pine bark medium which yielded 41 cuttings on average. This study demonstrated that the *P. patula* x *P. tecunumanii* (LE) hybrid is a feasible substitute for *P. patula* in both vegetative propagation systems, as it not only shows improved survival, through increased *F. circinatum* tolerance, but also improved productivity.**

Keywords: *Pinus patula*, *Pinus tecunumanii*, *Fusarium circinatum*, pine propagation, rooting, hedge mortality, disease tolerance, hydroponic, sand beds, polythene bags

Introduction

The emergence of the pine pathogen, *Fusarium circinatum* in South Africa in the early 1990s (Viljoen *et al*. 1994) has led to a reluctance in the local industry to continue to establish *Pinus patula* and *Pinus radiata*, which have been shown to be susceptible to this disease-causing agent. *Pinus patula* is the most important softwood species in the eastern regions of southern Africa, but exhibits low levels of tolerance to the pathogen (Viljoen *et al*. 1995, Coutinho *et al*. 2007, Wingfield *et al.* 1999, 2008, Mitchell *et al*. 2012a). Reports indicate that in South Africa, the problems associated with *F. circinatum* are primarily limited to nursery and field establishment (Viljoen *et al.* 1994, 1995, Crous 2005, Mitchell *et al*. 2012b) although the pathogen has also resulted in pitch cankers on large trees (Coutinho *et al*. 2007). In the Mpumalanga province Crous (2005) observed a 19% to 32% decrease in *P. patula* survival in compartments between November 2002 and March 2004 as a result of the pathogen. Considering these facts, *F. circinatum* has become one of the greatest threats to the pine forestry industry in South Africa (Wingfield *et al*. 2008, Mitchell *et al*. 2011). As a result of the disease related difficulties in propagating *P. patula* from seed or cuttings, coupled with declining establishment success and the need for repeated planting of newly established plantations, there has been pressure on the local forestry industry to reduce the deployment of *P. patula*. With considerable breeding resources invested in the improvement of growth and wood properties of *P. patula,* most large forestry companies in South Africa are understandably hesitant to abandon the species. One of the possible strategies for reducing the losses associated with *F. circinatum* is to make selections of more tolerant geographic races and families within *P. patula* (Viljoen *et al*. 1995, Hodge and Dvorak 2000, 2007, Mitchell *et al*. 2012b). Alternatively *P. patula* may be hybridised with more tolerant *Pinus* species (Roux *et al*. 2007, Mitchell *et al*. 2012c, 2013) which include *P. tecunumanii* and *P. oocarpa* (Hodge and Dvorak 2000, 2007, Mitchell *et al*. 2011, 2012c).

The production of hybridised, control-pollinated seed is a costly and logistically difficult activity, which often yields low amounts of viable seed (Sutton 2002). At this early stage, enough hybrid seed cannot be produced on the scale required to replace *P. patula* seedling production in South Africa. Therefore, it has been necessary to investigate the possibility of producing various *P. patula* hybrid replacements through vegetative propagation of rooted cuttings. This methodology is common and has been developed across many pine species including *Pinus radiata* in Australia, Chile and New Zealand (Allsop 1950, Cameron 1968, Thulin and Faulds 1968, Cameron and Thomson 1969, Fielding 1970, Bolstad and Libby 1982, West 1984, Menzies *et al*. 2001, South *et al*. 2005); *Pinus taeda* in Argentina and the southern United States (Frampton and Hodges 1989, Greenwood and Weir 1995, Goldfarb *et al*. 1998, Hamann 1998, Frampton *et al*. 2000, 2002, Foster *et al*. 2000, Murthy and Goldfarb 2001, LeBude *et al*. 2004, Rowe *et al*. 2002a, Gocke 2006); *Pinus patula* in South Africa (Mitchell 2005); *Pinus sylvestris* in Sweden (Högberg 2005); *Pinus pinaster* in Spain (Majada *et al*. 2010, Martínez-Alonso *et al*. 2012); *Pseudotsuga menziessi* in the Western United States (Ritchie *et al*. 1992, 1993) and *Picea abies* in Germany (Clair *et al*. 1985).

Commercial scale vegetative propagation of the *Pinus* genus has been successfully implemented in *P. radiata* in New Zealand (Cameron and Thomson 1969; Menzies *et al*. 1986, 2001, Talbert *et al*. 1993), Australia (Fielding 1970, Talbert *et al*. 1993) and Chile (Lewis *et al*. 1993); *P. taeda* in the United States and Argentina (Ritchie 1991, Talbert *et al*. 1993); *P. pinaster* in in Spain, Portugal and

France (Ritchie 1991, Majada *et al*. 2010) and Australia (Talbert *et al*. 1993) and *P. elliottii* x *P. caribaea var. hondurensis* hybrid in South Africa (Bayley and Blakeway 2002) and Australia (Trueman 2006). Rooted cuttings are the primary means of *P. radiata* deployment in Australia and New Zealand (Ritchie 1996).

While work has also been conducted on the vegetative propagation and establishment of *P. patula* as a pure species in South Africa (Mitchell *et al*. 2005a, 2005b, South and Mitchell 2006), the production of *P. patula* cuttings has ceased due to the high levels of *F. circinatum*-related mortality observed in hedge stock plants and cuttings since 2008.

The most common cutting production systems in South Africa are hedges established in polythene bags with composted pine bark as the growing medium. Fertiliser application is most commonly though granulated N:P:K (Nitrogen: Phosphorous: Potassium) or controlled release fertilisers applied to the top layer of the media. Hydroponic systems were introduced in pine vegetative propagation as sand beds in Brazil in the later part of the 1990s (de Assis *et al*. 2004). These are normally in the form of raised beds approximately 25 cm deep, 50 cm wide, and ten m long, and are filled with washed sand (McNabb *et al*. 2002). They are supplied with water and nutrients through a drip irrigation system. This system has not, however, been tested on pine species in South Africa. While the drip irrigation system has been shown to be suitable for the production of *Pinus taeda* and *P. elliottii* (de Assis *et al*. 2004) there is no literature available on its suitability for *P. patula, P. elliottii* x *P. caribaea*, or *P. patula* hybrids.

With little information specifically published on the vegetative propagation of *P. patula* hybrids, this study investigated *P. patula* and various pine hybrids in two vegetative propagation systems. The main objectives were to test productivity in rooting efficiency and the number of rooted cuttings that could be successfully produced from the hybrids, and to determine whether the hybrid hedge stock plants would survive better than the *F. circinatum*-susceptible pure species in each of the propagation systems. A further outcome of this research was to investigate family differences in survival and rooted cutting production of hedges specifically within *P. patula* and the *P. patula* x *P. tecunumanii* (LE) hybrid cross as this hybrid had been identified as a likely replacement for the pure species.

Materials and Methods

Trial Design

This trial series comprised two experiments within the same nursery and in close proximity; a taxa production experiment (1) and a propagation system experiment (2).

The production experiment (1) was implemented using a randomised complete block (RCB) design of 23 family treatments (across eight hybrid and pure taxa), with five replications. Each family plot, within replication, comprised 20 hedges. A total of 2300 hedges were included in this experiment and were planted in black polythene bags with composted pine bark growing media. The hedge container experiment (2), established in an adjacent growing tunnel but with the same seedling stock as the first experiment, used a split plot design with two hedge system types (hydroponic sand beds and polythene bags with composted pine bark growing media) as the main plots and 23 family treatments (across eight hybrid and pure taxa) as subplots. Subplots consisted of row plots with ten hedges each. Only two replications were completed due to limited availability of the experimental sand beds. A total of 1200 hedges were included in this experiment.

Plant material

Various pure and hybrid taxa (Table 1) and families within each, were sown into a 90 ml insert volume containerised system in January 2008 at the Sappi Shaw Research Centre located near Howick, South Africa (S29°28.53' E30°10.75'). The taxa were selected to represent a range of predicted *F. circinatum* tolerance (Hodge and Dvorak 2000; Mitchell *et al*. 2011). These included *P. patula* family selections (open and control-pollinated sources), *P. patula* hybridized with *P. greggii* var. *greggii*, *P. greggii* var. *australis, P. tecunumanii* (from low and high elevation ecotypes) and *P. oocarpa*, as well as *P. elliottii* x *P. caribaea* var. *hondurensis*. The seedlings were grown in the nursery under plastic and 20% hail net and received fertigation at 1200 µS/cm twice a week, until they were approximately 8-months-old. They were then individually planted into 4.5 L polythene bags filled with composted pine bark, or planted into sand beds, and managed as hedged stock plants.

Table 2.1: Number of families and hedges of *P. patula*, *P. patula* hybrids and *P. elliottii* x *P. caribaea* var. *hondurensis*, established in two experiments testing their vegetative propagation potential, after DNA fingerprinting.

Taxa	Pollination	Number of Families	Number of Hedges
P. patula	OP	8*	458
P. patula	CP	5	628
P. patula	Self	1	6
P. patula x *P. greggii* var. *greggii*	CP	3	382
P. patula x *P. greggii* var. *australis*	CP	3	355
P. patula x *P. tecunumanii* (HE)	CP	3	410
P. patula x *P. tecunumanii* (LE)	CP	15**	703
P. patula x *P. oocarpa*	CP	4	418
P. elliottii x P. caribaea var. *hondurensis*	CP	5	140
Total		55	3500

HE = High Elevation provenances; LE = Low Elevation provenances; CP = controlled pollination; OP = open pollination. *While five open-pollinated *P. patula* families were initially included, fingerprinting resulted in eight families which could be included in the analysis. ** *P. patula* x *P. tecunumanii* (LE) families comprised five *P. patula* females, pollinated with a pollen polymix of five male parents resulting in 15 unique families which could be included in the analysis.

Hedge growing methods and rooted cutting production

In all cases a pine mini-hedge system was employed to produce juvenile shoots for vegetative propagation. Two differing containerised systems were used. The first method, used in both the hedge production experiment and hedge system experiment employed hedges raised in 4.5 L polythene bags filled with composted pine bark. Granular N:P:K (2:3:2) was applied at a rate of 10 g per bag every six months for the first two years, after which a 12 month controlled release fertiliser (Scott's Osmocote® Exact Standard High K – N:P:K 11:11:18) was applied annually. Irrigation of hedges was manual and by overhead Netafim SpinNet™ sprinklers, with shoulder distribution, delivering 200 L of water per hour. During 2011, hedges were briefly under-watered due to an operational error which had a noticeable impact on hedge survival and productivity. The bagged hedges in experiment 2 were hand watered during the experiment duration and did not experience the same water stresses.

The second hedge establishment method used a hydroponic sand bed system, as pioneered in Brazil (McNabb *et al*. 2002, de Assis *et al*. 2004), and found to be successful in the vegetative propagation of *P. taeda* and *P. elliottii*. Hedges were established in 3.0 m x 1.2 m sand beds at an espacement of 15 x 10 cm. Fertigation was applied once daily for 20 minutes at 09h00 at a concentration of between 1200 and 1500 µS/cm (15:6:13 N:P:K), via six lines of Netafim drippers (pressure compensated non-leaking, delivering 1.1 L per hour per dripper). Beds were flushed with pure water every one to two weeks, depending on the electrical conductivity (EC) of the leachate.

Rooted cuttings were produced between October 2008 and June 2012 from 3500 hedges. This consisted of harvesting suitable juvenile shoots, approximately 8 cm in length, from the hedges every two to three months and setting them in composted pine bark medium for rooting in a greenhouse. The Unigro 98 tray type that was used has a cavity volume of 90 ml (Mitchell *et. al.* 2005b). The greenhouse was an enclosed plastic tunnel with a wet wall and fan cooling system set to cool at 26℃ and with a bottom heating system set with a ci rculation temperature of 40℃. Cuttings were misted with Netafim Coolnet™ nozzles delivering 7.5 L of water per hour. Irrigation water was delivered at a pH of 6.8 and average electrical conductivity of 135 µS/cm.

Over the 45 month period a total of 23 shoot harvests were set. Three settings; 10, 11 and 13, were excluded from the data analyses because nursery irrigation system failures were experienced during rooting.

Disease screening and genotype confirmation

All dying hedge plants, over the duration of the experiment period, were collected and sent to the Tree Protection Co-operative Programme (TPCP) Diagnostic Clinic of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria for morphological and molecular (Schweigkofler 2004) confirmation of infection by *F. circinatum*.

Needle samples from a subset of 493 hedges across selected hybrid crosses and *P. patula* as well as all 714 hedges of *P. patula* x *P. tecunumanii* (LE) were submitted to the Forest Molecular Genetics (FMG) Programme at the University of Pretoria for DNA fingerprinting to confirm their hybrid status. The DNA fingerprinting technique used common microsatellite markers (SSR). Individuals not conforming to the expected taxa were excluded from the analysis.

Statistical analysis

The GenStat® (15th edition) statistical software package was used to analyse the data. Hybrid taxa effects were analysed using general analysis of variance for *F. circinatum* mortality, rooting percentage and rooted cutting data. There were eight taxa levels and seven replications. This data set was comprised of mean values per taxa per replication and experiment for all hedges in polythene bags in experiment 1 and 2, with blocking for replication nested within trial.

Family effects in *F. circinatum* mortality, rooting percentage and rooted cutting production were analysed using general analysis of variance data. There were 31 family levels and seven replications. This data set consisted of summarised values per taxa per replication and experiment for all hedges in polythene bags in experiment 1 and 2, with blocking for replication nested within trial. Due to genetic contamination within taxa, family effects were only analysed for *P. patula* x *P. tecunumanii* (LE), control-pollinated *P. patula* and open-pollinated *P. patula*.

Taxa by propagation system effects were analysed using analysis of variance for a split plot design for *F. circinatum* mortality, rooting percentage and rooted cutting data. There were two propagation system levels and eight taxa levels within each propagation system with two replications. This data set consisted of summarised values per taxa per replication in experiment 2, with blocking for propagation system (whole plots), taxa (split plots) and replication.

Results and Discussion

Hedge mortality in nursery

The mean hedge plant mortality, across replications, over the forty-five month duration of this study was 40%. *Fusarium circinatum* was confirmed to have accounted for almost half of the observed hedge losses with a mean mortality of 18.3% across the replications. Of the 1351 dying hedges submitted to FABI for pathogen confirmation between 2008 and 2012, 47% were found to be infected with *F. circinatum*, 9% with *F. oxysporum* and 8% with *Diplodia pinea.* In 35% of the hedges no pathogen could be identified. The presence of secondary pathogens and a high number of hedges without evidence of disease suggests that hedge maintenance in this experiment may have been suboptimal and hence should also be considered an important determinant of hedge survival.

In the first year of production only 0.6% of the initial number of hedges were lost to *F. circinatum*, this increased to 6.2% in the second year and 8.4% in the third. In the fourth year the mortality decreased to 1.9%. This trend was observed across all taxa and suggests that mortality through *F. circinatum* can be a function of time spent in the nursery. Whether this was a result of physiological changes with time or the build-up of inoculum requires further investigation. It is possible that the decrease in mortality seen in year four is a result of natural selection within the hedges for tolerance, or that structural maturation made it more difficult for infection to occur. Mortality, related to *F. circinatum*, was at its highest during summer, peaking in November (3.2%) and March (2.4%). December, January and February experienced 1.8%, 1.2% and 1.6% mortality respectively. Mortality decreased sharply during autumn (April and May) from 2.0% to 1.4% and was 0.7% by June. July and August (mid-winter) showed the lowest incidence of *F. circinatum* related mortality with 0.3% of the experiment lost in those months over the four year period.

The hedge mortality for *P. elliottii* x *P. caribaea* var. *hondurensis* and *P. patula* x *P. tecunumanii* (LE) due to *F. circinatum* was 7% and 6%, respectively, and significantly lower (p < 0.001) than all other taxa (Figure 1). The *P. elliottii* x *P. caribaea* var. *hondurensis* and *P. patula* x *P. tecunumanii* (LE) findings are consistent with the tolerance reported in previous studies, as Hodge and Dvorak (2000) found *Pinus* species such as *P. tecunumanii, P. caribaea* and *P. oocarpa* to be highly tolerant to *F. circinatum.* Steenkamp *et al.* (2012) found low elevation sources of *P. patula* x *P. tecunumanii* to be more tolerant of *F. circinatum* infection than the high elevation sources and *P. patula*. In field inoculation studies Roux *et al*. (2007) showed that *P. elliottii* x *P. caribaea* and *P. patula* x *P. oocarpa* hybrids were highly tolerant of the pathogen while *P. patula*, *P. greggii* var. *greggii* and their hybrids were highly susceptible.

In this study, mortality levels due to *Fusarium circinatum* in open and control-pollinated *P. patula* and the hybrids of *P. patula* and *P. tecunumanii* (HE), *P. greggii* var. *australis, P. greggii* var. *greggii* and *P. oocarpa* were not significantly different, ranging from 18.2 - 26.6% with a least significant difference, at the 5% level, of 10.7%. This observation differs from that seen in the greenhouse infection studies of Mitchell *et al*. (2013) which showed a significant range of tolerance across these hybrid taxa. According to Mitchell’s results, the hybrids of *P. patula* with *P. oocarpa* and *P. greggii* var. *australis* exhibited increased tolerance to *F. circinatum*.

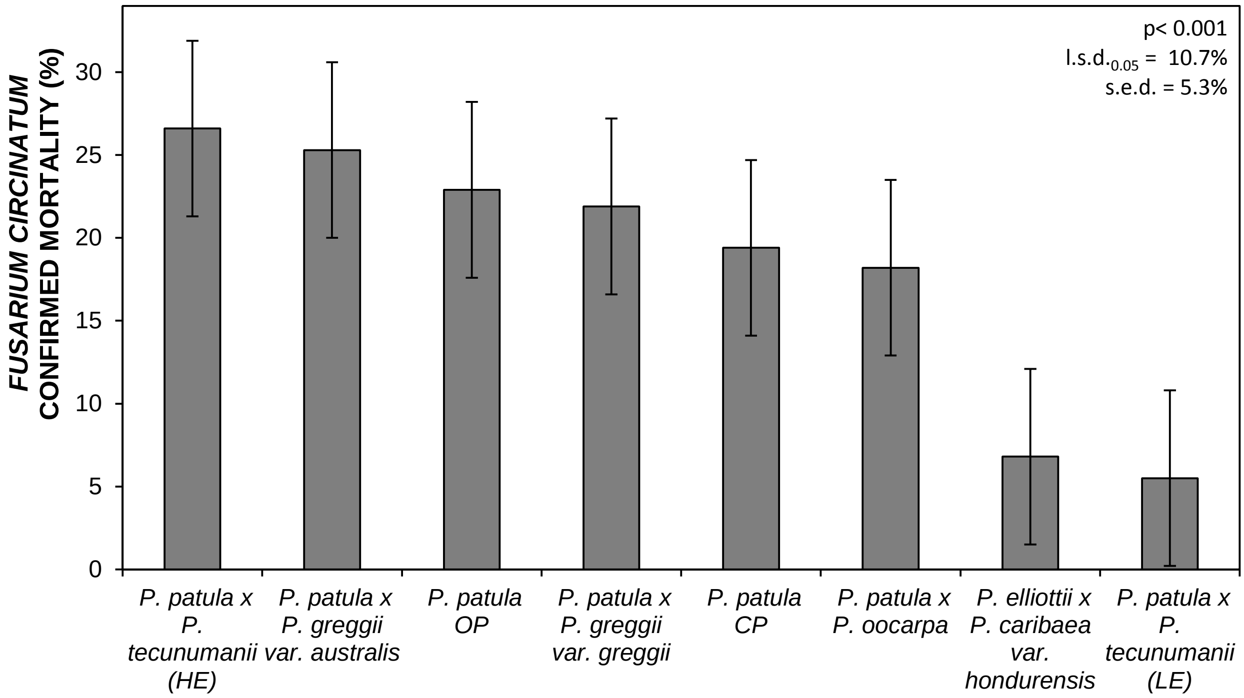

Figure 2.1: Hedge stock plant mortality per taxon that was positively linked to infection with *F. circinatum* in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. OP = open-pollinated; CP = controlled-pollinated; HE = High elevation; LE = Low elevation. Error bars represent the standard errors of differences of means. (For AVOVA outputs, table 2.3 in Appendix A refers)

DNA fingerprinting results for the *P. patula* hybrids (Table 2) indicated a high prevalence of *P. patula* x *P. patula* contamination within the samples of *P. patula* x *P. tecunumanii* (HE), *P. patula* x *P. oocarpa, P. patula* x *P. greggii* var. *australis and P. patula* x *P. greggii* var. *greggii*. While these specific contaminants were excluded from the analysis, not all hedges were fingerprinted and hence it is likely that a proportion of the remaining hedges were *P. patula* x *P. patula*. This would tend to explain the *P. patula* like tolerance, and lack of significant differences, observed between the pure and hybrid taxa. This observation highlights the importance of DNA fingerprinting hybrid plant material of highly related species that are difficult to distinguish morphologically. DNA fingerprinting of the *P. patula* x *P. tecunumanii* (LE) hedge plants revealed a low incidence of pure species contamination at 11%. All contamination in the *P. patula* x *P. tecunumanii* (LE) hedge plants could be excluded from the analysis as all hedges were fingerprinted for this taxa.

Table 2.2: *P. patula* contamination in hybrid taxa revealed through DNA fingerprinting of a selection of hedges from experiments 1 and 2.

Hybrid Taxa	Total hedges established	No. hedges submitted	*P. Patula* contamination %
P. patula x *P. greggii* var. *australis*	360	36	14
P. patula x *P. greggii* var. *greggii*	400	38	47
P. patula x *P. oocarpa*	520	168	61
P. patula x *P. tecunumanii* (HE)	460	157	32
P. patula x *P. tecunumanii* (LE)	712	712	11
	2452	1111	22

Due to the uncertainty around species purity, family mortality differences were only investigated for open and control-pollinated *P. patula* and for *P. patula* x *P. tecunumanii* (LE). Significant family effects (p < 0.001) in the hedge mortality rankings of *P. patula* families (open and control-pollinated) were observed (Figure 2) ranging from 8 - 44%. There were no significant differences in the mortality observed within *P. patula* x *P. tecunumanii* (LE) families, all of which were below 11%. Most of the control-pollinated *P. patula* families, which were selected for *F. circinatum* tolerance and growth (Nel *et al*. 2014), performed similarly to the *P. patula* x *P. tecunumanii* (LE) families, with mortality ranging from 10 - 20%, with the exception of one family (P4 x P8), which was significantly worse, with 38% mortality (Figure 2). Half of the open-pollinated *P. patula* families showed significantly higher *F. circinatum*-related mortality than the hybrid families.

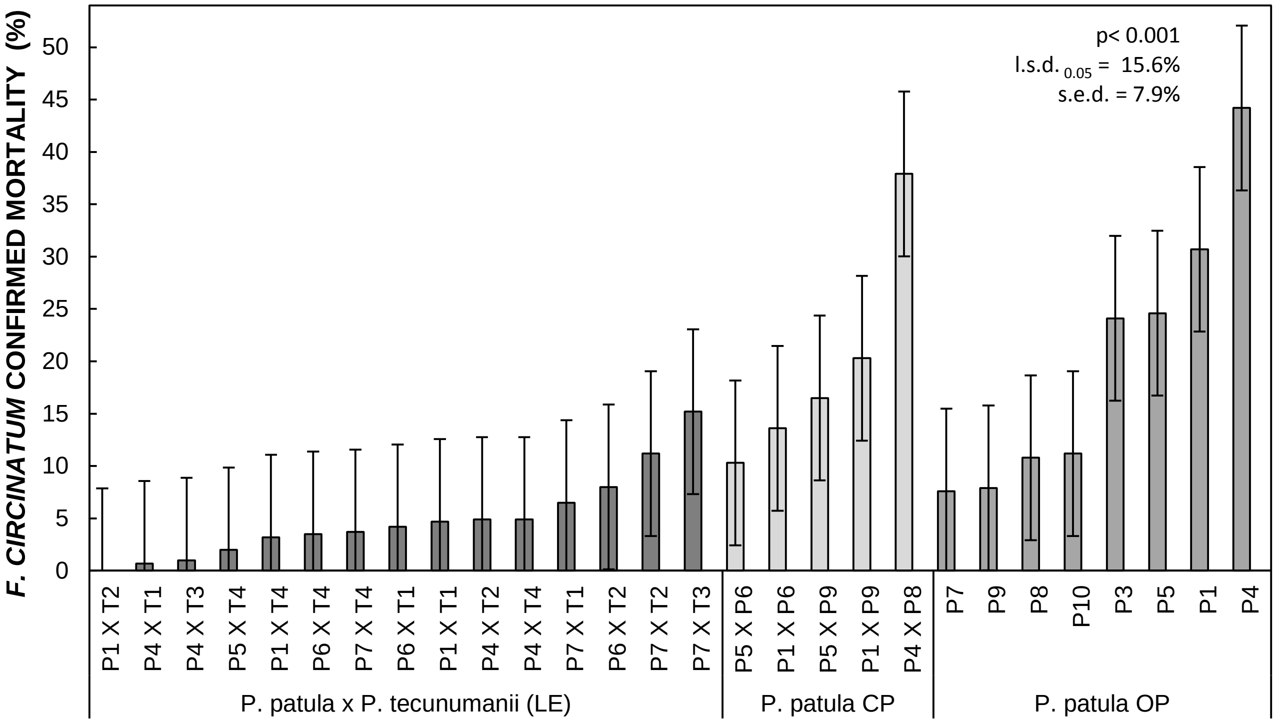

Figure 2.2: Hedge stock plant mortality for *P. patula* (controlled-pollinated and open pollinated) and *P. patula* x *P. tecunumanii* (LE) families that were positively linked to infection with *F. circinatum* in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. P = *P. patula* parent, T = *P. tecunumanii* parent. Error bars represent the standard errors of differences of means. (For AVOVA outputs, table 2.4 in Appendix A refers)

Fusarium circinatum host susceptibility differences at the family or provenance level have previously been reported (Hodge and Dvorak 2000, 2007; Mitchell *et al*. 2011, 2012c). Results from this study indicate the potential of selecting *P. patula* families that display increased tolerance to *F. circinatum* that could potentially be deployed as rooted cuttings. This supports the findings of Nel *et al.* (2014) who showed that artificial screening experiments could identify genetic variation among *P. patula* families for *F. circinatum* tolerance and provides evidence of the repeatability of those findings. *Pinus patula* x *P. tecunumanii* (LE) families showed consistently high tolerance to *F. circinatum* as indicated by the good survival of the hedges. This finding is consistent with greenhouse inoculation studies

(Mitchell *et al*. 2013) and is due to the high tolerance of the *P. tecunumanii* (LE) pollen parent (Mitchell *et al*. 2012b). Despite the relatively poor tolerance observed in the *P. tecunumanii* (HE) in this experiment, only three families were included in this study. High elevation sources of *P. tecunumanii* may show some opportunity for selection as the most tolerant provenances of *P. patula* show similar tolerance to the mean of the high elevation provenances of *P. tecunumanii* (Hodge and Dvorak, 2007).

Hedge productivity in nursery

Rooting in the *P. elliottii* x *P. caribaea* var. *hondurensis* hybrid was significantly better than in all other taxa with 84% rooting success. *Pinus patula* x *P. greggii* var. *australis* and *P. patula x P. tecunumanii* (LE) rooted at 70 and 69% respectively, which was significantly better ($p < 0.001$) than *P. patula* at 60% (Figure 3).

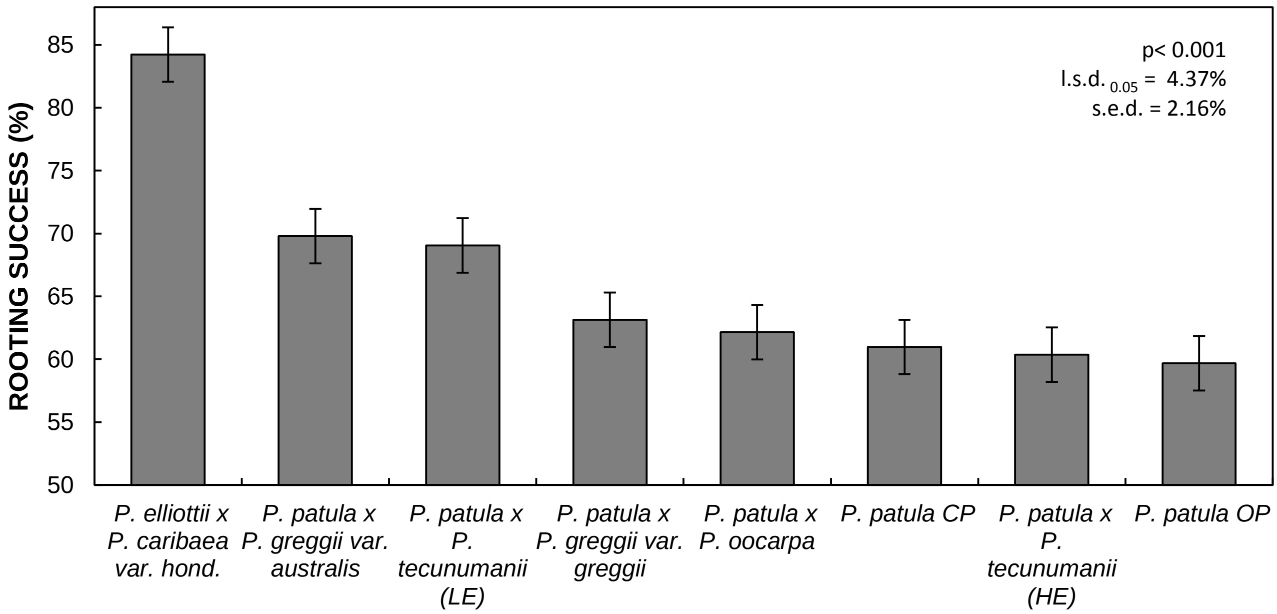

Figure 2.3: Mean rooting percentage of cuttings from pure and hybrid taxa over a 45 month period (20 settings) in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. Error bars represent the standard errors of differences of means. (For AVOVA outputs, table 2.5 in Appendix A refers)

There was no significant difference in the rooting of *P. patula* produced by either open or controlled-pollination. The rooting of *P. patula* families (open or control-pollinated) showed less variation than the *P. patula x P. tecunumanii* (LE) families and generally performed poorly (45 - 66%) compared to the *P. patula x P. tecunumanii* (LE) hybrid families (49 - 79%, Figure 4). The rooting results achieved in this study confirm that the *P. patula x P. tecunumanii* (LE) hybrid can be propagated by cuttings as successfully as other current commercial taxa such as *P. elliottii* x *P. caribaea* var. *hondurensis*.

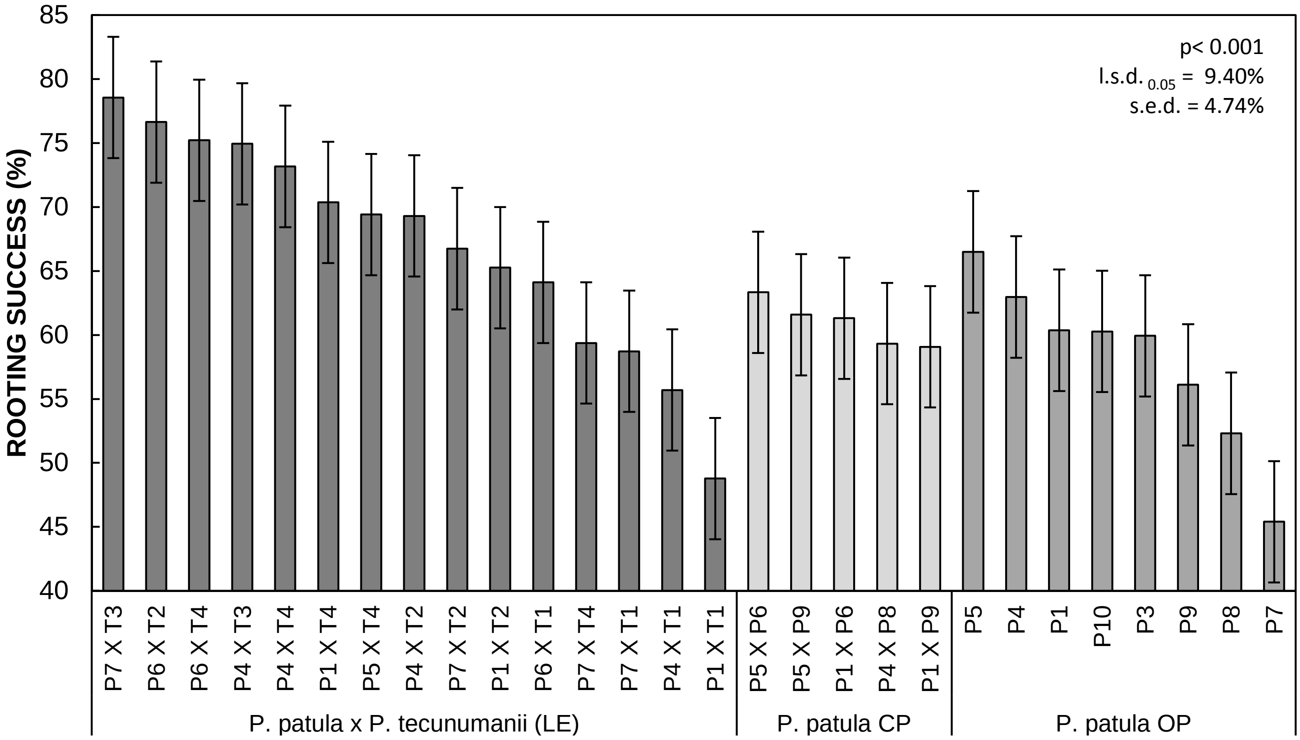

Figure 2.4: Mean rooting percentage of cuttings from *P. patula* (control-pollinated and open-pollinated) and *P. patula* x *P. tecunumanii* (LE) families in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. P = *P. patula* parent, T = *P. tecunumanii* parent. Error bars represent the standard errors of differences of means. (For AVOVA outputs, table 2.6 in Appendix A refers)

The number of rooted cuttings produced per hedge initially established, a combined measure of hedge survival effects as well as shoot production and rooting over the four year period, was 52 in the *P. patula* x *P. tecunumanii* (LE) hybrid, significantly higher (p<0.001) than the *P. patula* x *P. greggii* var. *australis* and *P. elliottii* x *P. caribaea* hybrids (41, Figure 5).

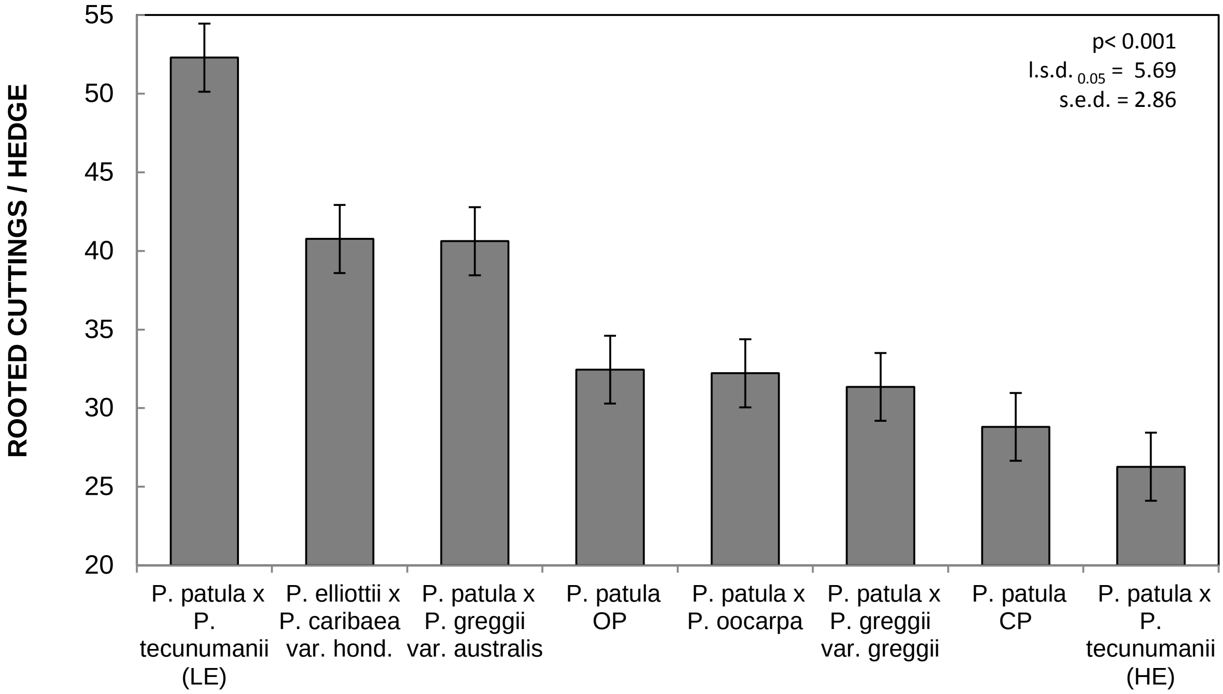

Figure 2.5: Number of rooted cuttings established per hedge from the pure and hybrid taxa over a 45 month period (20 settings) in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. Error bars represent the standard errors of differences of means. (For AVOVA outputs, table 2.7 in Appendix A refers)

There was no significant difference in the number of rooted cuttings produced in open-pollinated versus control pollinated *P. patula* at 33 and 29 respectively. The *P. patula x P. tecunumanii* (LE) hybrid showed considerable variation among families in the production of rooted cuttings, which ranged from 35 to 70 rooted cuttings per hedge (Figure 6). Although *P. patula* showed more consistent production, this was much lower than that of the hybrid, with families ranging between 20 and 42 cuttings per hedge.

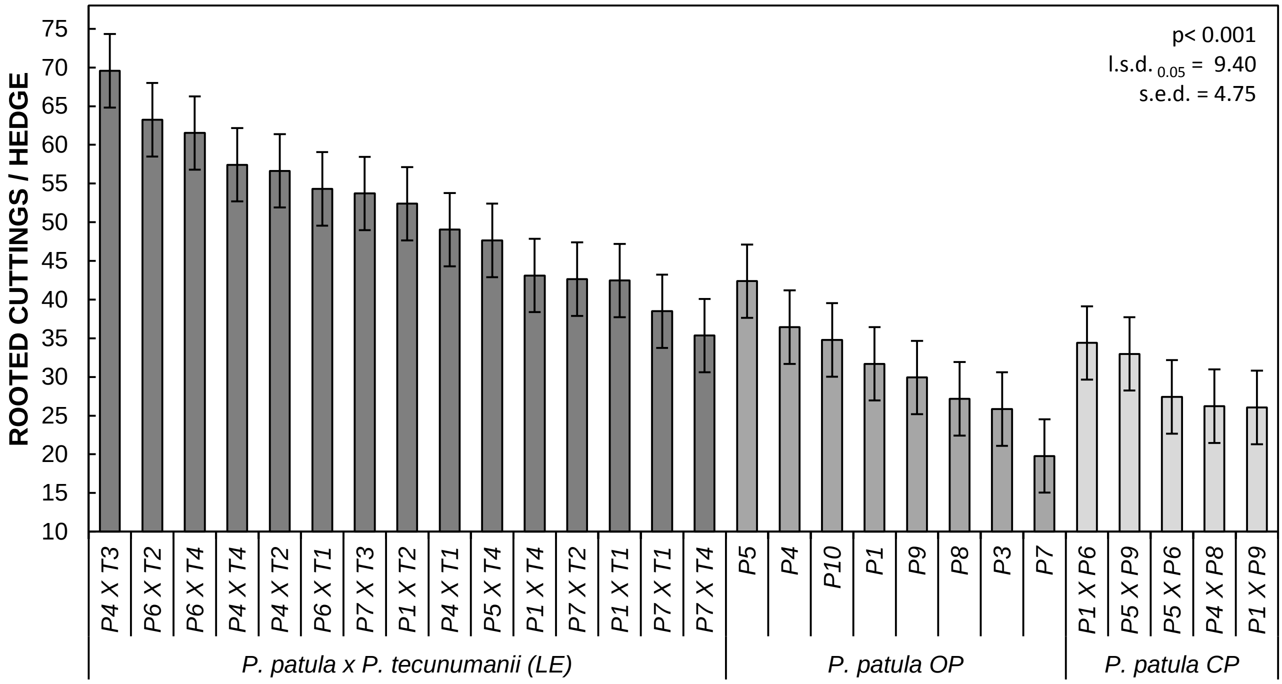

Figure 2.6: Rooted cutting production per family for *P. patula* (control-pollinated and open-pollinated) and *P. patula* x *P. tecunumanii* (LE) in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. P = *P. patula* parent, T = *P. tecunumanii* parent. Error bars represent the standard errors of differences of means. **(For AVOVA outputs, table 2.8 in Appendix A refers)**

Under ideal propagation conditions an average of 32 to 48 rooted cuttings per stock plant per year are common in *P. taeda* and *Pinus elliottii* x *P. caribaea* (Rocha and Niella 2001), while Martínez-Alonso *et al*. (2012) reported approximately 39 cuttings per hedge in the first years production season in *Pinus pinaster*. This may indicate that improvement in the propagation system employed in this study could have been possible where a similar number of rooted cuttings were produced over a four-year period. For the purposes of this experiment, harvesting was conducted approximately every two to three months whereas in a commercial environment this would have been every two to three weeks. Furthermore all hedge stock plants received very conservative nutrition in their first two years and a short drought event in their second year; this resulted in lower than optimal shoot production. The expected commercial production standard used by a South African company for *P. patula x P. tecunumanii* (LE), based on trial and commercial production figures, is 120 to 150 cuttings per established hedge over their life span of four years, which equates to an average of 2.5 to 3.1 cuttings per hedge per month.

Propagation system effect on F. circinatum *mortality and hedge productivity*

The propagation system type trial (experiment 2) exhibited only 9.5% *F. circinatum* related mortality. Propagation system had no effect on the frequency of *F. circinatum* related mortality with hedge mortality in sand beds versus bags being 9.4% and 9.6% respectively ($p = 0.920$). No significant taxa differences in *F. circinatum* related mortality were observed in this experiment ($p = 0.137$) but mortality ranged from 0% in *P. elliottii* x *P. caribaea* var. *hondurensis* to 15% in *P. patula* x *P. greggii* var. *greggii*. It is possible that the low incidence of *F. circinatum* related mortality in this experiment, combined with low replication could explain the lack of significant differences.

Propagation system had no significant effect on rooting success ($p = 0.511$) with rooting being 66% in cuttings from beds and 67% from bagged hedges, this is despite assumptions of better nutritional management in a hydroponic system (de Assis *et al*. 2004). It has been reported that increased nitrogen application rates have resulted in higher rooting percentages in *P. pinaster* and *P. taeda* (Rowe *et al*. 2002a, 2002b, Martínez-Alonso *et al*. 2012). Significant taxa differences in rooting success were observed in this experiment ($p < 0.001$). The *P. elliottii* x *P. caribaea* hybrid, at 85%, remained the best rooter followed by the *P. patula x P. tecunumanii* (LE) and *P. patula* x *P. greggii* var. *australis* hybrids at 76% and 73%, respectively. There was no significant difference in the rooting of the remaining *P. patula* and *P. patula* hybrids which ranged from 57% to 62%. There was no interaction effect on rooting between the propagation system and species (p = 0.349).

The propagation system had a significant effect on the number of rooted cuttings produced per hedge ($p < 0.001$, $LSD_{0.05} = 2.7$ cuttings) with an average of 55 cuttings per hedge from hedges in beds and 41 from bagged hedges (Figure 7).

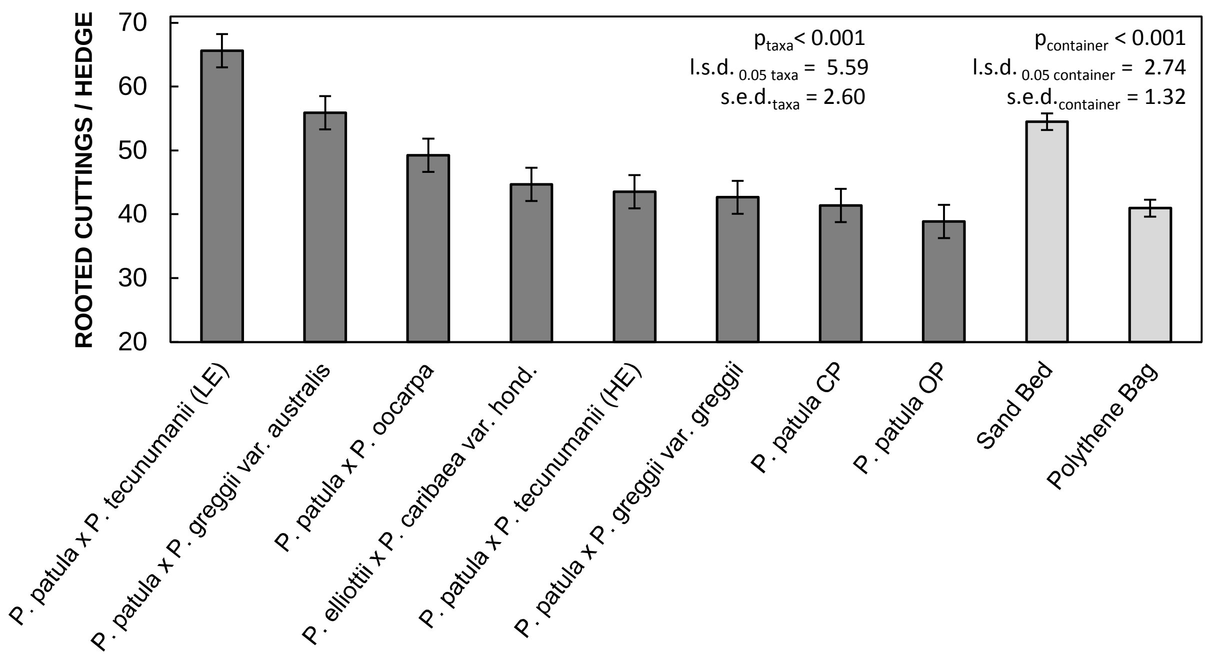

Figure 2.7: Number of rooted cuttings established per hedge from the pure and hybrid taxa and two propagation systems, in experiment 2, over a 45 month period (20 settings). Error bars represent the standard errors of differences of means. (For AVOVA outputs, table 2.9 in Appendix A refers)

There was no interaction between propagation system and species (p = 0.664), all taxa were significantly more productive in the sand bed system. A possible explanation for this is that the hydroponic system provides a significant advantage to the hedge plants by providing well-balanced nutrition (de Assis *et al*. 2004) which is delivered with consistency. Increased nitrogen has been shown to increase the number of shoots produced in various pine species (Rowe *et al*. 2002b, Martínez-Alonso *et al*. 2012). As in the analysis of the taxa in polythene bags in both experiments, *P. patula x P. tecunumanii* (LE) in experiment 2 in both polythene bags and sand beds combined showed the highest number of rooted cuttings with an average of 66 produced per established hedge. *Pinus patula* x *P. greggii* var. *australis* also remained highly ranked with 56 cuttings per hedge. The number of rooted cuttings from *P. elliottii* x *P. caribaea* was similar at 45 rooted cuttings, but only ranked fourth in this analysis and was not significantly better than *P. patula*.

Conclusion

This study demonstrates that the survival of *P. patula* hedge plants can be significantly improved by selecting families that are more tolerant to *F. circinatum*. Further improvements can be made by hybridizing *P. patula* with *P. tecunumanii*, particularly the low elevation source. Other potential hybrid partners have been identified and could provide increased tolerance to *F. circinatum*, and potentially undergo family selection, but this was difficult to detect in this trial due to *P. patula* x *P. patula* contamination. This highlights the importance of identity confirmation by DNA fingerprinting before making selections based on tolerance. There was no further improvement in tolerance through the selection of specific families within the 15 *P. tecunumanii* (LE) families tested in this study, which all showed high tolerance.

P. patula x *P. tecunumanii* (LE) showed significantly higher rooted cutting production per established hedge, than all other hybrid crosses of *P. patula,* due to its excellent rooting and shoot production ability. Furthermore, the variation in rooting and shoot production among families of this hybrid indicates that this can be further improved. The adoption of a hydroponic hedge maintenance system provided a significant increase in hedge productivity and could be used to further improve the vegetative propagation of all pine species under investigation.

The results of this study indicate that *P. patula x P. tecunumanii* (LE) is highly tolerant to *F. circinatum and* can easily be deployed as rooted cuttings. It therefore, offers a viable alternative to *P. patula* in a vegetative propagation system.

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Appendix A

ANOVA output tables for data represented in Figures 2.1 – 2.7

Table 2.3: ANOVA outputs for hedge stock plant mortality per taxon that was positively linked to infection with *F. circinatum* in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance (Figure 2.1).

Variate:
Average_of_Fusarium_circinatum_mortality

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	6	0.251804	0.041967	4.26	
rep.*Units* stratum					
Row_Labels	7	0.314171	0.044882	4.56	<.001
Residual	42	0.413432	0.009844		
Total	55	0.979407			

Table 2.4: ANOVA outputs for hedge stock plant mortality for *P. patula* (controlled-pollinated and open pollinated) and *P. patula* x *P. tecunumanii* (LE) families that were positively linked to infection with *F. circinatum* in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance (Figure 2.2).

Variate: Average_of_Fusarium_circinatum

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
rep stratum	6		0.67790	0.11298	5.21	
rep.*Units* stratum						
family_group	27		2.46946	0.09146	4.21	<.001
Residual	126	(36)	2.73420	0.02170		
Total	159	(36)	5.37424			

Table 2.5: ANOVA outputs for mean rooting percentage of cuttings from pure and hybrid taxa over a 45 month period (20 settings) in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance (Figure 2.3).

Variate: Average_of_rooting_%_excl_fails (bags only, no beds)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	6	328.96	54.83	3.35	
rep.*Units* stratum					
Species_hybrid	7	3331.25	475.89	29.05	<.001
Residual	42	688.1	16.38		
Total	55	4348.31			

Table 2.6: ANOVA outputs for mean rooting percentage of cuttings from *P. patula* (control-pollinated and open-pollinated) and *P. patula* x *P. tecunumanii* (LE) families in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance (Figure 2.4).

Variate: Average_of_rooting_%_excl_fails

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
rep stratum	6		1088.43	181.41	2.30	
rep.*Units* stratum						
family_group	27		12629.07	467.74	5.94	<.001
Residual	118	(44)	9293.75	78.76		
Total	151	(44)	18484.49			

Table 2.7: ANOVA outputs for number of rooted cuttings established per hedge from the pure and hybrid taxa over a 45 month period (20 settings) in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance (Figure 2.5).

Variate: Average_of_total_rooted_cuttings

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	6	796.16	132.69	4.77	
rep.*Units* stratum					
Species_hybrid	7	3520.15	502.88	18.09	<.001
Residual	42	1167.83	27.81		
Total	55	5484.14			

Table 2.8: ANOVA outputs for rooted cutting production per family for *P. patula* (control-pollinated and open-pollinated) and *P. patula* x *P. tecunumanii* (LE) in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance (Figure 2.6).

Variate: Average_of_total_rooted_cuttings

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
rep stratum	6		3561.13	593.52	7.52	
rep.*Units* stratum						
family_group	27		33165.90	1228.37	15.57	<.001
Residual	118	(44)	9308.98	78.89		
Total	151	(44)	37656.51			

Table 2.9: ANOVA outputs for number of rooted cuttings established per hedge from the pure and hybrid taxa and two propagation systems, in experiment 2, over a 45 month period (20 settings) (Figure 2.7).

Variate: Average_of_total_rooted_cuttings

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	36.87	36.87	1.34	
prop method stratum	1	1466.39	1466.39	53.23	
rep.prop method stratum	1	27.55	27.55	2.25	
rep.prop method.*Units* stratum					
Species	7	2243.93	320.56	26.15	<.001
Residual	21	257.43	12.26		
Total	31	4032.17			

Variate: Average_of_total_rooted_cuttings_propagation method

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	36.87	36.87	2.48	
Species stratum	7	2243.93	320.56	21.54	
rep.Species stratum	7	104.16	14.88	1.23	
rep.Species.*Units* stratum					
prop method	1	1466.39	1466.39	121.65	<.001
Residual	15	180.82	12.05		
Total	31	4032.17			

CHAPTER 3

THE SURVIVAL AND GROWTH OF *PINUS PATULA* X *PINUS TECUNUMANII* ROOTED CUTTINGS IN RESPONSE TO VARYING MORPHOLOGICAL PLANT QUALITY PARAMETERS IN A FIELD STUDY IN SOUTH AFRICA.

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The survival and growth of *Pinus patula* x *Pinus tecunumanii* rooted cuttings in response to varying morphological plant quality parameters in a field study in South Africa.

CM Ford*[1, 2], NB Jones[1], JW Crous[1] and PWC Chirwa[2]

[1]Sappi Forests, Shaw Research Centre, PO Box 473, Howick, 3290, South Africa

[2]Forest Science Postgraduate Programme, Department of Plant Production and Soil Science, University of Pretoria, Pretoria, 0002, South Africa

** Corresponding author, email: craig.ford@sappi.com*

The potential gains from high quality planting stock include optimum stocking and volume growth, consequently most commercial conifer growing nurseries make use of morphological grading systems. This paper tested the morphological plant quality specifications for *Pinus patula* x *Pinus tecunumanii* (low elevation, LE) rooted cuttings required for optimal survival and growth after planting. Based on this study the ideal raising period for *P. patula* x *P. tecunumanii* (LE) cuttings, grown in a 90ml cavity is 10 months from setting. The ideal height for cuttings was 28-32cm and the ideal root collar diameter range was 3.5mm - 4.5mm. The root plug was optimal when no growing medium fell off the plug when extracted from the insert; the root plug was firm but not hard and the plug was well colonised with a high proportion of thin brown roots. It was optimal to have at least three or more actively growing white root tips present and visible evidence of ectomycorrhizae. Needles in the dark mid-green to dark green range were shown to be optimal.

These recommendations were based on findings from a single trial site that experienced good planting conditions and good rainfall. As a result, the effects of significant water stress on the survival and growth of these cuttings was not adequately assessed and would require further testing. It did however show that even under ideal planting conditions cutting age, height, root collar diameter, needle colour, root plug integrity and the number of white roots all had a significant effect on survival and growth of cuttings a year after field establishment.

Keywords: *Pinus patula*, *Pinus tecunumanii*, *Plant Quality, Cutting Morphology, Post Establishment Survival*

Introduction

Many factors can affect plant survival and subsequent growth, these include genetic variability, plant quality, stock type, handling and transportation of plants and silvicultural practices at planting (Mason 2001; Pinto et al. 2011). To provide the best possible chance of survival at planting it is important that the highest quality plants are used (Bernier et al. 1995; Sharma et al. 2007). It is the responsibility of nurseries to ensure that high quality plants are supplied to end users. For the purposes of this study, plant quality is described as a plant's ability to survive and grow after planting (Mattsson 1996). The potential gains from high-quality planting stock, in combination with good silvicultural practices in forestry, are additive and lead to optimum stocking as well as volume growth (South et al. 2005a). It is thus essential to characterise plant quality to ensure establishment success (Duryea 1985; Rose et al. 1990; Mattsson 1996; Puttonen 1996; Tsakaldimi et al. 2012). Establishment success relates primarily to site conditions and transplant stresses which are most commonly associated with water stress in forestry (Grossnickle and Folk 1993; Close et al. 2005). In order for plants to overcome transplant stress and successfully establish after planting it is essential that the root system can meet the transpirational demands of the shoot system. It is therefore important to have immediate root growth and colonisation of the soil as well as sufficient soil contact and good root permeability (Burdett 1990). It is thus necessary for plants to have the functional physiological processes required for root growth and development (Grossnickle and Folk, 1993).

Evidence of the predictive ability of morphological plant quality parameters on field performance has been widely documented (Thompson 1985; Menzies et al. 1986; Mexal and Landis 1990; Long and Carrier 1993; Puttonen 1996; South 2000;

Mitchell et al. 2005b; Mitchell et al. 2005a; Wilson and Jacobs 2006; Ritchie and Landis 2010; Grossnickle 2012). Most commercial conifer growing nurseries make use of morphological grading systems (Ritchie et al. 1992) which are still seen to have value (Puttonen 1996), and are often preferred over physiological measures as they are more easily and quickly implemented. By quantifying and scoring plant quality, one is able to predict the initial field survival capacity, as well as the growth potential of batches produced by the nursery (Grossnickle and Folk 1993).

The most commonly used morphological parameters in conifer plant quality assessment are shoot height, root collar diameter (RCD), root and shoot biomass, root:shoot ratios and the quantity and colour of foliage (Puttonen 1996). Specifications for container grown pine species were published by the mid 1990's and included height, RCD, the height:RCD ratio or quality exponent (Hodgson and Donald 1980) or sturdiness ratio (Ritchie and Landis 2010) and plants were grouped by size (Johnson et al. 1996). Seedling height, RCD and sturdiness ratio have all been shown to be important determinants of pine field performance (Donald 1992; Bayley and Kietzka 1997). The important question regarding morphological plant measures for particular species grown in particular container types is; what height, RCD and root volumes are required for optimum survival on different sites (Pinto et al. 2011).

The pine pathogen, *Fusarium circinatum*, represents a significant threat to the commercial production of *Pinus patula* seedlings and cuttings in Southern Africa (Mitchell et al. 2011; Mitchell et al. 2012a). In response to this threat, research has been conducted on the development of *F. circinatum*-tolerant *P. patula* hybrids and the vegetative propagation of these hybrids (Mitchell et al. 2012b; Mitchell et al. 2013; Ford et al. 2014). Various hybrid options have been identified to replace the *P.*

patula as a pure species (Roux et al. 2007). Work on the vegetative propagation of *P. patula* x *P. tecunumanii* (low elevation, LE), as a potential replacement of the pure species has been reported (Ford et al. 2014) but no plant quality specifications have been investigated and formalised.

This study aimed to test the morphological plant quality specifications required for optimal survival and growth after planting of *P. patula* x *P. tecunumanii* (LE) rooted cuttings, grown in a 90ml container volume

Materials and Methods

Plant material

Cuttings between 2 and 23 months of age, from time of setting, were selected from *P. patula* x *P. tecunumanii* (LE) hybrid families to establish a plant quality field trial. The cuttings harvested were 8cm in length and were set to a depth of 2cm and were rooted, without the use of hormones, in a misthouse for 2 months before being transferred to the greenhouse for hardening. Cuttings were raised in Unigro 98 seedling trays consisting of individual 90ml inserts, at the Sappi Shaw Research Centre located near Howick, South Africa (S29°28.53 ' E30°10.75'), using composted pine bark (12mm) growing medium. Irrigation water was delivered at an average pH of 6.8 and average electrical conductivity of 135 µS/cm. Weekly fertigation of 15:6:13 N:P:K (+micronutrients) water soluble fertilizer was applied to cuttings at an average electrical conductivity of 1300 µS/cm. A total of 20 hybrid families were represented across replications and treatments. Where possible, the same 49 clones were included in each treatment plot across all replications in order to remove any genetic effects in the trial. Clone position was randomised within plots.

Field Trial

Rooted cuttings between 2 to 23 months of age were grouped into five age treatments, cuttings 2-4, 5-7, 10-12, 14-16 and 19-23 months old, and planted in a randomised complete block design in 7 by 7 tree plots and with 6 replications. Plant quality measures where thus assessed across 1470 *P. patula* x *P. tecunumanii* (LE) cuttings. The trial site was a good quality warm temperate site in the KwaZulu-Natal Midlands with and altitude of 809m above sea level, mean annual precipitation of 1195mm and mean annual temperature of 17.6℃. The t rial was planted at the end of the planting season on 12 April 2012. The site received 150mm of rain during April 2012 and 154mm during May 2012. The total rainfall over the first year after planting was 1626mm, which was above average for the site. Each cutting also received one litre of water at planting.

Measurements

In order to ensure a wide enough selection for height and RCD, age was used as the grouping factor for this study. Before trial establishment, plant quality parameters were measured and recorded for individual cuttings, which were then tracked into field where subsequent survival, height growth and RCD growth was recorded at 1 year. The cutting quality parameters included in this study were; plant age, height, RCD, needle colour, root plug colonisation, visual presence of ectomycorrhizae and number of visible white root tips.

Height and Root Collar Diameter

Height was measured to the nearest half centimetre using a ruler and was measured from the stem-substrate interface to the tip of the apical bud. Root collar diameter was measured using digital callipers at the stem-substrate interface.

Needle Colour

A twenty-category colour scale, with decreasing intensity of greenness, was developed for this trial. Plants of varying degrees of greenness where photographed and the dominant colour selected and used in a colour chart. Colours were grouped in four main categories; dark green, mid green, light green and pale green/yellow-brown, with five sub-categories in each (Refer to Table 3 in Appendix C for RGB values). Each cutting was placed on the colour chart and assigned a score according to its matching greenness, where a value of 1represented the darkest green and 20 reflected the least green cuttings and those that were yellow/brown.

Root Plug Integrity

A novel twenty category scale with increasing colonisation of root plugs, varying from a score of 1, where no roots were visible to 20 where they were highly root bound, was developed for this trial. A visual aid with photographs and a descriptive key was used to assign scores to the level of colonisation for each cutting (Refer to Table 4 in Appendix D for the key developed to differentiate cuttings based on root plug integrity). Scores between 10 and 14 represent what may be regarded as ideally colonised.

Ectomycorrhizal Presence

The presence of ectomycorrhizae was visually assessed and scored in four categories; either absent, visible in less than a third of the plug, visible in between a third and two thirds of the plug and visible in more than two thirds of the plug. Evidence of ectomycorrhizae was indicated by the presence of bifurcate root nodules and hyphal growth.

White Root Tips

The number of visible white root tips was counted for each of the four quadrants of the root plug for each cutting established in field.

Field Height and Root Collar Diameter measurement

Tree heights were assessed, to the nearest centimetre, from ground level to the tip, using a height rod designed for this purpose. Root collar diameter was measured in millimetres, to two decimal places, at ground level using digital callipers

Statistical Analysis

The statistical software package GenStat® (Version 15) was used to analyse all data. Linear and non-linear regression analyses for standard curves were assessed for use in the analysis of survival, height growth and root collar diameter growth for each of the measured morphological parameters represented by continuous data. This excluded the analysis of ectomycorrhizae presence and prevalence, which were discreet data. All data points with fewer than ten individuals making up the mean for that data point were excluded from the analysis or grouped. The regression model with the highest percentage variance accounted for was selected. When the percentage variance accounted for was similar across regression models, the most parsimonious curve was selected. Table 1 in Appendix B shows the detail of the regression models fitted to the data for Figures 1a to 3f. The analysis of variance was performed for a randomized complete block design to identify differences in the four ectomycorrhizae categories. Data was summarised by the four categories and by replication and analysis run as a balanced design.

Results and Discussion

Plant Age Specifications

The age of the cuttings in this study had a significant impact on survival (P<0.001), where cuttings aged between 3 and 6 months of age showed an increase in survival from 65% to 90% (Figure 1a). In a containerised plant production system an age window exists where plants display optimum root biomass for the container size and type. Prior to this, as shown in the cuttings younger than 6-months-old (Figure 1a), the plant root plug has not fully colonized the growing medium, which could then lead to loss of plug integrity and root damage at planting and ultimately to mortality after planting. There is also increased probability, as a result of pre-existing shoot biomass from setting, that the cuttings' root biomass may not yet be sufficient to support the transpirational needs of the shoot biomass once planted out. Cuttings older than 6 months of age all survived equally well at 94%. This was an unexpected result as one might envisage older, more root bound plants not to survive as well.

Plant age, irrespective of root plug status can affect field performance as roots become suberized with age and are less able to take up water, which is particularly important after planting when active moisture uptake is essential in ensuring plant survival (MacFall et al. 1991; Wells and Eissenstat 2003; Mitchell et al. 2005b; South and Mitchell 2006). In this trial it appears that a combination of applying water at planting, together with good rains after planting and an unusually wet winter period meant that none of the plants experienced a drought stress event. This was supported by the high survival (89%) after one year.

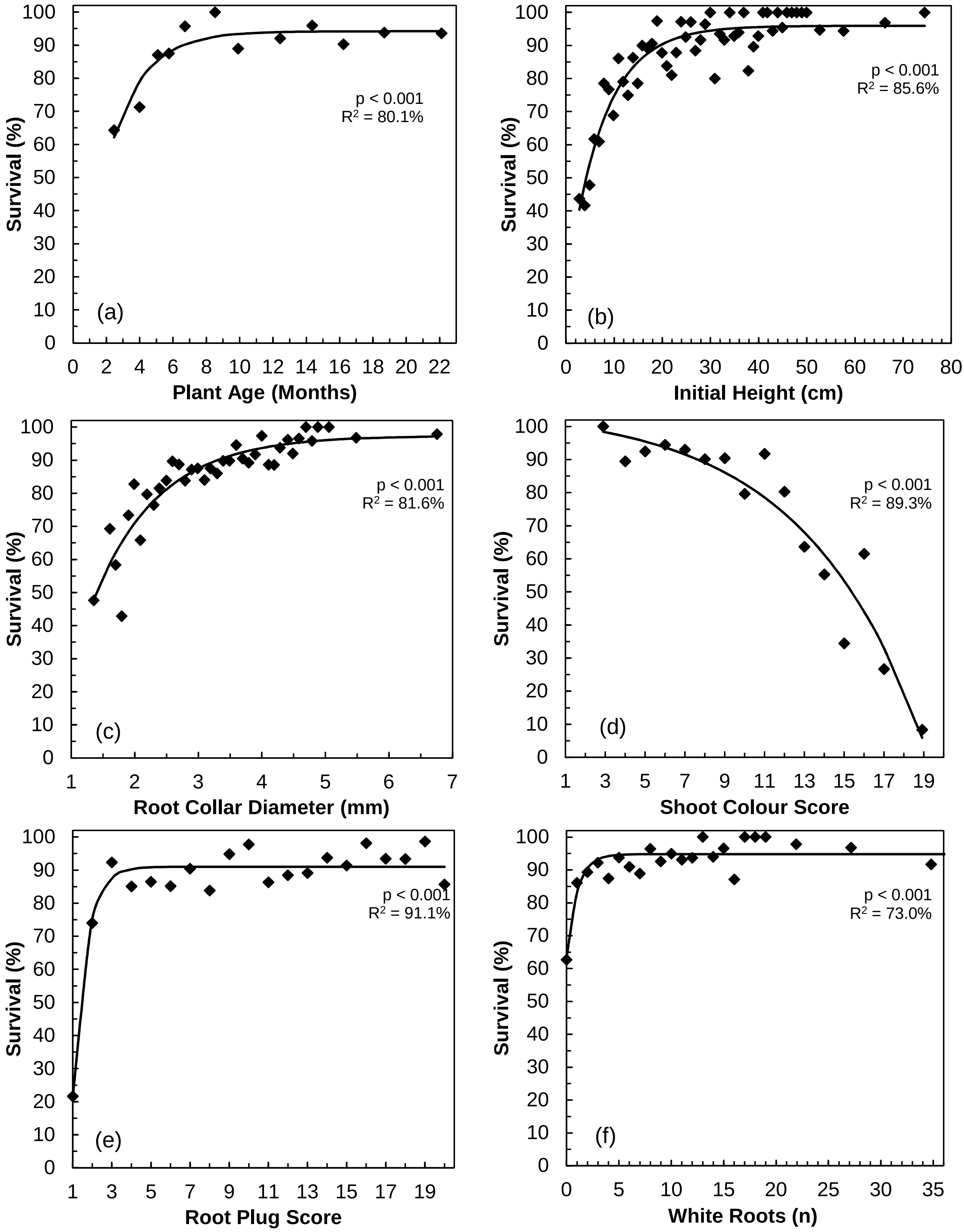

Figure 3.1: The effect of plant age (a), initial height (b), root collar diameter (c), leaf colour (d), root plug integrity (e) and the number of actively growing white root tips (f) on survival of *Pinus patula* x *Pinus tecunumanii* (LE) rooted cuttings one year after establishment in field.

Plants older than the ideal age window would not only tend to be suberized but also root bound. In this case the cuttings may show poorer field growth as well as basal sweep and windthrow in trees 1 to 2 years after planting. Any mortality associated with root defects would thus tend to become more significant with increasing age and height. The relationship between cutting age and height increment a year after planting (Figure 2a) was not significant (P = 0.229) but age did have a significant effect (P < 0.001) on RCD increment (Figure 3a). Cuttings between 3 and 10 months of age showed an increase in growth, from 13mm to 22mm. Plants older than 10 months of age all showed the same RCD increment of 22mm.

With optimal survival being reached between 8 and 10 months of age, and no increase in RCD growth after 10 months of age, this data shows that the ideal raising period for *Pinus patula* x *Pinus tecunumanii* (LE) cuttings, grown in a 90ml cavity, under the nursery conditions described, is 10 months from placing. Any additional time in the nursery would not increase first year growth or survival potential but would increase the unit cost per cutting through increased nursery inputs.

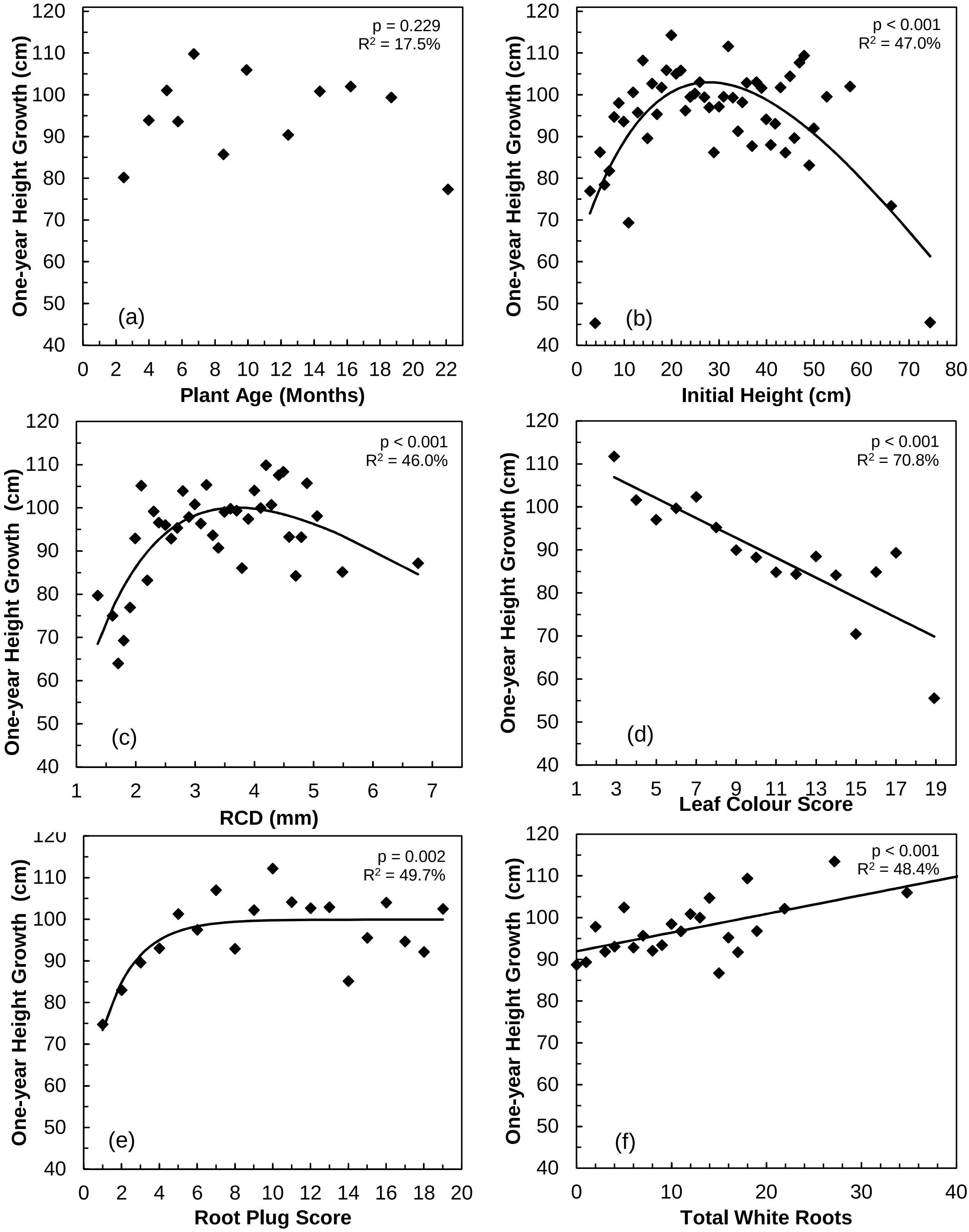

Figure 3.2: The effect of plant age (a), initial height (b), root collar diameter (c), leaf colour (d), root plug integrity (e) and the number of actively growing white root tips (f) on height growth of *Pinus patula* x *Pinus tecunumanii* (LE) rooted cuttings one year after establishment in field.

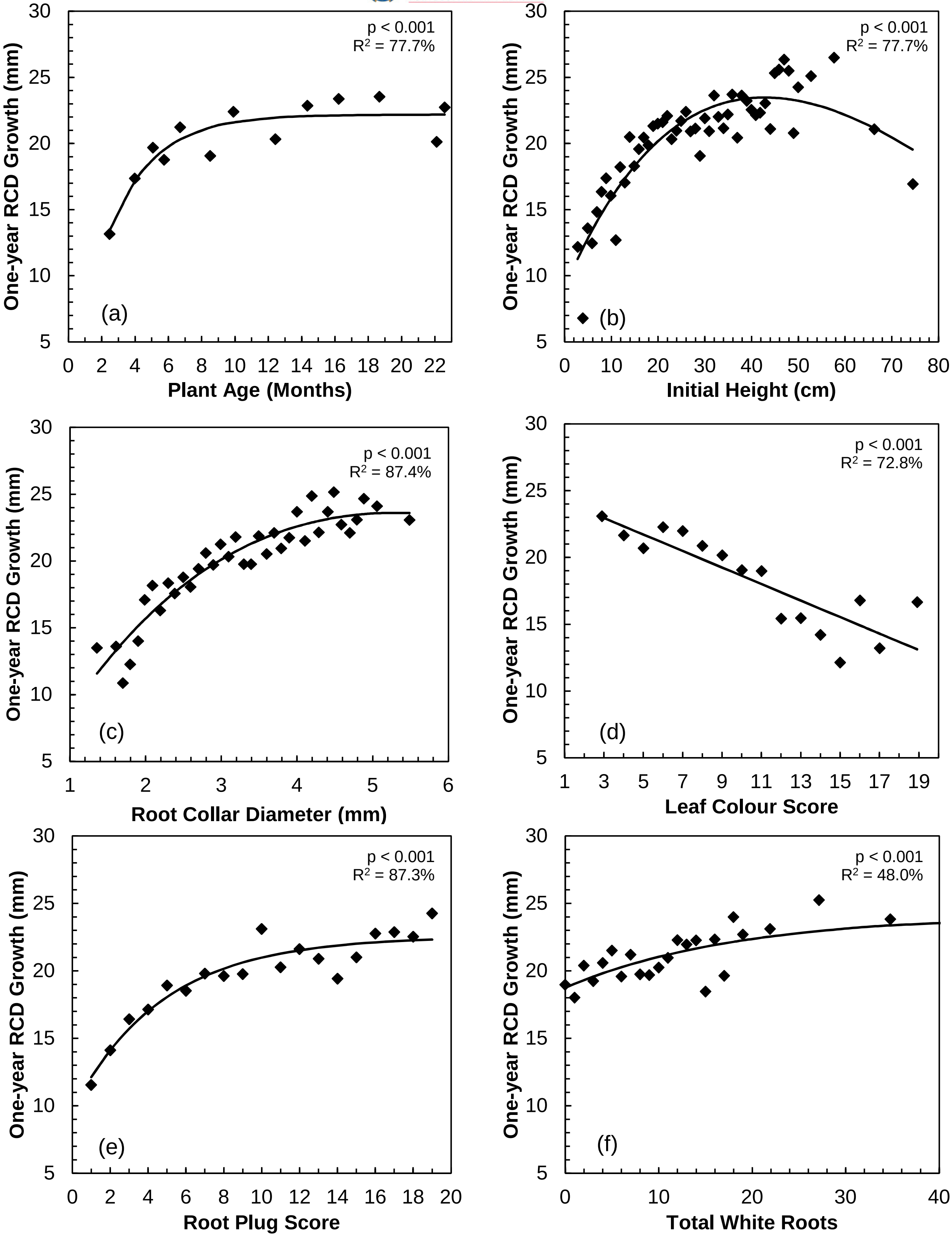

Figure 3.3: The effect of plant age (a), initial height (b), root collar diameter (c), leaf colour (d), root plug integrity (e) and the number of actively growing white root tips (f) on root collar diameter growth of *Pinus patula* x *Pinus tecunumanii* (LE) rooted cuttings one year after establishment in field.

Plant Size Specifications

Raising the ideal plant size for a specific container type/volume is important in both growth and survival (Johnson et al. 1996; Zwolinski and Bayley 2001; Ortega et al. 2006). Plants that exceed the size recommendations have a high probability of being root bound, while plants below the recommended size would tend not to have fully colonised the plug. Seedling height, RCD and sturdiness ratio are important determinants of field performance (Donald 1992; Bayley and Kietzka 1997; Ritchie and Landis 2010). The heights and RCD of plants raised in a containerised nursery system are of vital importance as container size determines the limit of biomass which can be supported.

Height

Cutting height had a significant ($P < 0.001$) effect on survival at one year after planting. Cuttings between 3cm and 20cm showed increasing survival from 44%to 90% (Figure 1b). Cuttings 20cm and taller survived, on average, at above 90%. The poorer in survival associated with the smaller plants may relate to their age and consequently to their poorer root development as previously discussed under the effects of plant age. It is unlikely that this is an effect of competition for light, which can limit survival through decreased photosynthetic carbon gain (Johnson and Smith 2005) as regular weeding within the trial took place. Cuttings taller than 30cm, up to 74cm, showed equally good survival of 95%.

The idea of increasing survival with taller plants is supported by previous work on bareroot *P. taeda* seedlings (Tuttle et al. 1987) but only on sites with little environmental stress and when root mass is not limited by container size. As shoot biomass increases it can result in an inability of the root system to supply sufficient water to meet the transpirational demands of the plant thus increasing susceptibility to transplant stress (Grossnickle 2005). Shoot height is a good measure of not only the transpirational area of plants but also their photosynthetic potential (Grossnickle 2012), as it is correlated with the number of needles (Ritchie and Landis 2010). Larger plants, in the absence of a transpirational deficit, would therefore be expected to show increased survival through increased photosynthetic potential. Sites where drought stress is likely to occur, taller plants are at greater risk of water stress and of mortality (Larsen et al. 1986; Boyer and South 1987; Rose and Gleason 1993; Stewart and Bernier 1995; McTague and Tinus 1996). This can, however, be mitigated if the plants have the ability to quickly develop new roots (Grossnickle 2012). It is thus likely that the cuttings in this trial experienced very low water stress during the first months after establishment, were still actively growing and were able to quickly develop roots thereby avoiding drought stress later in the winter season. The poor survival of plants shorter than 20cm, despite the good planting conditions, highlights the sensitivity of these plants to transplant stresses.

Initial cutting height also had a significant effect ($P < 0.001$) on cutting height increment at 1 year after planting (Figure 2b). In a hyperbolic relationship, cuttings between 3cm and 28cm in height showed a rapid change in shoot growth from 70cm to 100cm in the year. Cuttings with heights between 32cm and 74cm showed a decrease in height growth from 100cm to 61cm in the year. Shoot height has been shown to be a good measure of photosynthetic ability and also potential growth (Mexal and Landis 1990) and it is generally accepted that larger seedlings tend to produce greater amounts of new shoot biomass (Thiffault 2004; Grossnickle 2005; Grossnickle 2012). This holds true in the current study for plants shorter than 30cm where the container size is not a limiting factor. As the plants increased further in height from 32cm, the negative impacts of becoming increasingly root bound were manifested on growth (Figure 2b). These effects will be discussed in detail later in relation to the effects of root plug colonisation. It is also important to consider that under water stressed conditions larger plants can show reduced growth (Baer et al. 1977; Stewart and Bernier 1995; Khan et al. 1996; Lamhamedi et al. 1996), which would also tend to limit the size of plant suitable for planting.

Initial cutting height had a significant effect ($P < 0.001$) on cutting RCD increment at one year after planting (Figure 3b). In a hyperbolic relationship, cuttings between 3cm and 34cm in height increased in RCD growth from 11mm to 22mm in the year. Cuttings with heights between 40cm and 74cm showed a decrease in RCD growth from 22mm to 19mm in the year. Root collar diameter has been shown to be correlated with root biomass (South and Mitchell 2006). These data therefore suggest that not only did 1 year stem diameter increase with increasing initial cutting height, up to 34cm, but that the root biomass also increased. Decreasing stem diameter growth in cuttings taller than 40cm at planting suggests that physiological limitations have most likely been imposed on root growth of cuttings with heights in this range.

With optimal survival being reached at 28-30cm, maximum height increment between 28-30cm and maximum RCD growth at 32-34cm, the ideal height for *Pinus patula* x *Pinus tecunumanii* (LE) cuttings, under good planting conditions, was 28-32cm.

Root Collar Diameter

Root collar diameter had a significant effect (P < 0.001) on survival of cuttings 1 year after planting (Figure 1c). Survival rapidly improved from 47% to 87% as RCD increased from 1.3mm to 3.0mm. Between 3.0mm and 4.0mm, survival increased from 87% to 93%. Cuttings with RCD's greater than 4.0mm and less than 6.8mm survived equally well at approximately 95%. South and Mitchell (2006) showed the thresholds for being root bound, through the use of a Root Bound Index (RBI), and that their subsequent effect on survival can vary between species. A RBI (RCD in mm/cavity volume in cc x 100) of 3.6 was found to be the maximum threshold for *P. patula* cuttings before decreases in survival were observed, while *P. elliottii* x *P. caribaea* cuttings only showed a survival decrease with indexes between 5 and 6, with 7 being identified as the critical value. The maximum RCD measured in the present trial (6.8mm, raised in a 90ml cavity), translates to a RBI of 7.2 but no impact on survival was observed. Being root bound did not, therefore, seem to impact on establishment success in this trial, based on 1 year survival measurements.

Root collar diameter did however have a significant (P < 0.001) effect on shoot height increment a year after planting (Figure 2c). Cuttings with RCD's between 1.3mm and 2.5mm showed an improvement in height increment from 68cm to 94cm. Between RCD's of 2.5mm and 3.5mm, height growth increased from 94cm to 100cm. Height growth decreased from 100cm to 95cm between RCD's of 3.5mm and 5.0mm respectively. Between RCD's of 5.0mm and 6.8mm, height growth decreased further from 95cm to 87cm. The initial RCD also had a significant (P < 0.001) effect on the growth in RCD over the year (Figure 3c). Between initial RCD's of 1.3mm and 3.5mm, the growth in RCD improved from 11.5mm to 22mm. Between

3.5mm and 4.5mm RCD growth only increased slightly to 23 mm and after 4.5mm initial RCD there was no further improvement in RCD growth.

Root collar diameter, also commonly referred to as 'calliper', is a common tool used in measuring plant quality (Duryea 1984; Ritchie 1984; Thompson 1985; Grossnickle et al. 1988; Mexal and Landis 1990; McGrath and Duryea 1994; Sloan 1996; Frampton et al. 2002; Sanchez et al. 2003; Rose and Ketchum 2004; Rolando and Little 2005; South and Mitchell 2005; VanderSchaaf and South 2005; South et al. 2005b; Haase 2007; Haase 2008; Landis et al. 2010; Landis 2011) and hence in predicting survival and growth potential. Many studies show, through outplanting performance, that RCD is often the best measure of plant quality (Ritchie and Landis 2010).

With optimal survival being reached by a RCD of 4.0mm – 4.5mm, maximum height increment at 3.5mm and maximum RCD growth between 4.0mm – 4.5mm, these data show that the ideal RCD range for *Pinus patula* x *Pinus tecunumanii* (LE) cuttings, was 3.5mm - 4.5mm.

Plant health – Leaf colour

Needle colour had a significant ($P < 0.001$) effect on cutting survival at 1 year after planting (Figure 1d). Survival was negatively correlated with colour score (decreasing greenness), in a non-linear relationship. An average colour score of 2.8 resulted in 100% survival. Between dark to mid-mid green cuttings survival declined to 90% and between mid to light mid green survival declined to 80%. Within the light green category, survival decreased from 78% to 53%, while in the yellow green- light brown categories, survival decreased from 44% to 8%. The colour of the foliage gives an indication of the nutritional status of the plants and of their photoprotection ability (Close et al. 2005). Needles in the pale green, yellow-green and light brown ranges indicate lower chlorophyll content and vigour than dark green foliage (Ritchie and Landis 2010). Plant chlorophyll content, measured as 'greenness' in this study, can be used as an indicator of nitrogen status (Mattsson 1996). Needles which are deep green are generally indicative of high levels of nitrogen, while those in the light green to yellow range are indicative of low nitrogen levels and even nitrogen deficiency. Nitrogen in the deficient to low range can reduce drought resistance (Van Den Driessche 1991) and in some cases nitrogen levels which are too high can also reduce drought resistance in seedlings (Pharis and Kramer 1964; Etter 1969). Nitrogen status can therefore be used as an estimate of plant quality (Mattsson 1996). These data suggest that even in the absence of drought stress, the nutrient status of *Pinus patula* x *Pinus tecunumanii* (LE) cuttings can affect their ability to successfully establish.

Needle colour score had a significant (P < 0.001) effect on height increment 1 year after field planting (Figure 2d). There was a linear decrease in height growth from 104cm, in the plants scoring an average of 2.8, to 70cm in those scoring an average of 18.9. A similar trend was observed in the RCD increment over the 1 year period where growth showed a significant linear decrease with decreasing 'greenness' (Figure 3d). Root collar diameter growth decreased from 23mm, in the plants scoring an average of 2.8, to 13mm in those scoring an average of 18.9.

The colour of cuttings and the nutrient status this infers, is thus of great importance to both survival and growth in *Pinus patula* x *Pinus tecunumanii* (LE) cuttings even in the absence of drought stress at planting. It is likely that high nutrient reserves allow the cuttings to quickly develop new roots and rapidly colonise the soil. To optimise survival and growth in *Pinus patula* x *Pinus tecunumanii* (LE) thus requires dark green cuttings.

Root Plug Integrity

Root plug integrity had a significant ($P < 0.001$) effect on survival. Root plug integrity is an important factor to consider in plant quality as the degree to which roots have colonised their root plug is a good indicator of absorptive root surface (Thompson 1985). Plugs with no visible roots at extraction, scoring 1 on the root plug integrity scale, showed extremely poor survival of 20% (Figure 1e). Cuttings with very few roots visible and not able to hold any growing medium, scoring 2 on the root plug integrity scale, survived at 74%, while those scoring 3 on the scale (slightly more roots visible but still not able to hold any growing medium) had a further improved survival of 87%. Poor root development may be responsible for the plant not meeting the transpirational demands of the shoot system during adverse climatic conditions after planting (Burdett 1990). All plugs where the root mass was able to hold at least some growing medium, scoring between 4 and 20 on the root plug integrity scale, survived equally well at 91%. Good survival in plugs which were not optimally colonised can be explained by the good planting conditions that resulted in low transplant stresses through transpirational demands. Root plug integrity had a significant ($P = 0.002$) effect on height growth at 1 year after establishment (Figure 2e). Height growth rapidly improved from 75cm in category 1 to 97cm in category 6 (where little medium fell off the plug when extracted, but there was not yet a dense matting of fine roots and the plug was very soft with, mostly, white roots). Between scores of 7 and 20, no further improvement in height growth was observed and all cuttings grew equally well with an increase of 100cm. Root collar diameter growth was also significantly ($P < 0.001$) impacted by root plug status (Figure 3e). Between scores of 1 and 7, RCD increment improved from 12mm to 20mm respectively and between scores of 7 and 14 RCD growth continued to improve but only slightly to 22mm. No further increase in RCD growth was observed for root plug scores between 14 and 20.

Root plugs are classed as root bound when the plants have grown too large for their container, resulting in dense matting and tangling of the root system (Ritchie and Landis 2010). Under these circumstances the plug is generally hard with many roots visible on the outside of the plug, a high proportion of which are relatively thick brown roots. When root plugs become over colonised (or root bound) pine seedlings and cuttings show a reduction in their ability to produce new roots after planting. This can lead to reduced plant survival and growth after outplanting (South and Mitchell 2006). In this study, cuttings falling into the 16 to 20 score category would be classified as root bound yet no impact on survival was observed after a year in field. It is, however, still possible that this condition could cause decreased stability in the longer term (Lindström and Rune 1999), which may lead to mortality, with trees being blown over, as a result of poorer root structure.

Despite the effect of good planting conditions on the survival of relatively poorly colonised plugs, this study showed that even under ideal planting conditions plugs must have sufficient root mass to retain some growing medium in order to survive. Planting poorly developed root plugs would expose cuttings to greater survival risk under droughty conditions and should thus be avoided. With optimum height increment reached by a plug score of 7, and optimum RCD increased only reached by a plug score of 14, the recommendation from this study would be to dispatch plants in category 14. In this category, no medium fell off the plug when extracted, the root plug was firm but not hard and was well colonised with a high proportion of thin brown roots. While no negative impacts on survival and growth were observed for scores above 14, there would be disadvantages with regards to nursery space and costs in retaining plants for longer than required. There would also be increased risk of longer term growth and survival impacts.

White Root Tips

The presence of white, actively growing, root tips is important in regeneration success as many root tips, evenly spread on the surface of the plug, facilitates uniform and intense root growth after planting (South and Mitchell 2005). The number of white root tips per plug, had a significant effect on survival ($P < 0.001$) (Figure 1f). Where no white growing tips were visible survival averaged only 63%. The presence of a single visible white root increased survival to 86%, while a second and third white root tip improved survival further to 89% and 92%, respectively. No further improvement in survival was observed with four or more visible growing tips, with survival remaining at approximately 95% (Figure 1f). The number of white roots showed a significant ($P < 0.001$) positive relationship with height growth (Figure 2f). This linear relationship showed increased height growth, after a year, from 91cm where no white roots were visible to 106cm where thirty five white roots were visible (Figure 2f). The number of white roots also had a significant ($P < 0.001$) effect on RCD growth. Between zero and sixteen visible white roots, RCD growth increased from 19mm to 22mm (Figure 3f). Where between sixteen and thirty five white roots were visible, RCD only increased a further 1mm to 23mm. These results confirm that the presence of actively growing root tips on the outside of the plug is important in the successful establishment of *Pinus patula* x *Pinus tecunumanii* (LE) cuttings.

While three or more visible tips are sufficient to optimise survival, there are growth gains from having more visible white root tips.

Ectomycorrhizae colonisation

The average ectomycorrhizae prevalence scores measured in this study were positively correlated with root plug colonisation scores ($P < 0.001$, $R^2 = 93.9\%$). This reflected the initial difficulty of observing signs of ectomycorrhizae in the young, poorly developed plugs, with few visible roots, and the later effect of increased time for inoculation and hyphal growth in the nursery of the older, well- and over-colonised root plugs. To effectively test the specific impact of ectomycorrhizae prevalence on survival and growth of cuttings, root plug colonisation was used as a covariate in the analysis of variance performed. Even with plug colonisation as a covariate, the absence of ectomycorrhizae had a significant ($P < 0.001$) effect on survival of the *Pinus patula* x *Pinus tecunumanii* (LE) cuttings at one year after establishment, where adjusted survival was 68% versus between 89% and 93% in those which had evidence of ectomycorrhizae (Table 2).

Table 3.2: The effect of ectomycorrhizal presence on survival, height growth and root collar diameter growth in *Pinus patula* x *Pinus tecunumanii* (LE) rooted cuttings, adjusted for root plug colonisation as a covariate, 1 year after field planting.

	Ectomycorrhizae presence in plug (%)						
Variate	**0**	**<33**	**33-67**	**>67**	**P**	**s.e.d of means**	**l.s.d.**
Survival	67.6	93.4	92.1	88.6	<.001	5.2	11.2
Height increment (cm)	96.4	98.7	92.8	91.9	0.413	5.5	11.8
RCD increment (mm)	18.2	19.4	19.3	20.7	0.199	0.9	2.0

* Significance at the 0.05 level

The presence of ectomycorrhizae is usually visible as grey to white fungus on the root plug and/or where the tips of roots are swollen or branched (bifurcate). Beneficial mycorrhizal fungi, naturally colonising most horticultural and forestry plants, can increase disease resistance and enhance water and nutrient uptake as well as amino acid assimilation (Linderman 1993; Cuny 1995; Ekblad and Wallander 1995; Davies 2000; Taylor et al. 2004). There were no significant differences in the survival of the three levels of ectomycorrhizae presence. While research has shown that mycorrhizal associations can improve plant productivity (Johnson et al. 1997), the prevalence of ectomycorrhizae in the *Pinus patula* x *Pinus tecunumanii* (LE) cutting plugs did not have a significant effect on height (P = 0.413) or RCD (P = 0.199) increase during the year, when the effect of root plug colonisation was excluded. Combined presence versus absence, using plug integrity as a covariate did, however, show significant differences (P = 0.048, l.s.d. = 2.55mm) in RCD growth. Roots without visible presence of ectomycorrhizae grew 17.5mm while those with grew 20.1mm in the year.

Conclusion

In this study all phenotypic measures showed significant effects on both cutting survival and growth after planting. The largest differences in survival, in order of magnitude, were observed in needle colour, root plug score and cutting height. The largest differences in height growth, in order of magnitude, were observed in initial height, RCD and needle colour. Initial height, RCD, root plug score and leaf colour all showed large differences in RCD increment over the 1 year period.

While first year differences in growth are important to note and may manifest into longer term growth differences, some research has shown that these initial differences are temporary and their predictive ability for growth decreases with time. This is especially true on mesic sites as the limitations of the container environment are eclipsed by the effects of the field conditions and plant genetics (Pinto et al. 2011). The 1 year growth results from this study allow us to postulate where the possible of growth effects may occur, but in order to make accurate predictions of the effects of *Pinus patula* x *Pinus tecunumanii* (LE) cutting morphology on long term growth, further measurements are required throughout the rotation. Survival at 1 year, however, represents a good estimate of establishment success and the findings of this study can be used to predict survival of *Pinus patula* x *Pinus tecunumanii* (LE) cuttings.

Based on the data from this study, the ideal raising period for *Pinus patula* x *Pinus tecunumanii* (LE) cuttings, grown in a 90ml cavity under the nursery conditions described, and planted under favourable conditions on a warm temperate site, was 10 months from setting. The ideal height for these cuttings was 28-32cm and the ideal RCD range was 3.5mm - 4.5mm. The root plug integrity recommendation would be to dispatch plants where: no medium falls off of plug when extracted; the root plug is firm but not hard; the plug is well colonised with a high proportion of thin brown roots; there are at least three or more actively growing white root tips and there is visible evidence of ectomycorrhizae. In addition, needles must be in the dark mid-green to dark green range.

With this study being completed on a single site where high survival was obtained as a result of through good establishment conditions and planting practice, the recommendations made from these data require further validation across multiple sites, and under conditions of moisture stress moisture stress, to provide greater accuracy. It was also noted that the effects of plant age, height and RCD on survival and growth showed very similar relationships and it is likely that some co-linearity exists. It is therefore difficult to estimate the relative importance of each of the morphological parameters without further analysis accounting for the multiple co-linearity.

Plant quality parameters, as described in this study, only provide a predicted estimate of a plant's ability to survive and grow under normal conditions and not a guarantee of survival under any conditions (Grossnickle 2012). Adverse planting conditions and practices can easily compromise these predictions.

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Appendix B

Table 3.1: Formulae and level of significance of linear and non-linear standard curves used in the regression analysis of Figures 1a to 3f.

Figure number	Response variate	Explanatory variate	Formula for curve	F pr.	r^2	se
1a	1 year survival	Cutting age	Y = 94.2 + (-1.068*(0.6137**X))	<.001	80.1	4.65
1b	1 year survival	Initial height	Y = 95.93 + (-0.8062*(0.8749**X))	<.001	85.6	5.50
1c	1 year survival	Initial RCD	Y = 97.46 + (-1.85*(0.378**X))	<.001	81.6	5.76
1d	1 year survival	Needle colour	Y = 105.45 + (-0.0437*(1.1796**X))	<.001	89.3	9.12
1e	1 year survival	Plug integrity	Y = 91.01 + (-3.058*(0.2273**X))	<.001	91.1	4.88
1f	1 year survival	No. of white roots	Y = 94.83 + (-0.3143*(0.375**X))	<.001	73.0	4.03
2a	Height increment	Cutting age	Y = 89.06 + (-0.0045*(1.493**X)) + (0.93*X)	0.229	17.5	9.16
2b	Height increment	Initial height	Y = 183.9 + (-121.6*(0.9581**X)) + (-1.579*X)	<.001	47.0	9.99
2c	Height increment	Initial RCD	Y = 143.9 + (-166.4*(0.493**X)) + (-8.56*X)	<.001	46.0	7.96
2d	Height increment	Needle colour	Y =-2.311 + (113.59*X)	<.001	70.8	7.13
2e	Height increment	Plug integrity	Y = 99.91 + (-46.5*(0.57**X))	0.002	49.7	6.52
2f	Height increment	No. of white roots	Y =0.4472 + (91.94*X)	<.001	48.4	5.30
3a	RCD increment	Cutting age	Y = 22.183 + (-21.5*(0.6955**X))	<.001	77.7	1.37
3b	RCD increment	Initial height	Y = 53.2 + (-44.1*(0.973**X)) + (-0.374*X)	<.001	77.7	1.87
3c	RCD increment	Initial RCD	Y = 63 + (-64*(0.776**X)) + (-4.3*X)	<.001	87.4	1.29
3d	RCD increment	Needle colour	Y =-0.6177 + (24.8*X)	<.001	72.8	1.82
3e	RCD increment	Plug integrity	Y = 22.553 + (-12.87*(0.8102**X))	<.002	87.3	1.14
3f	RCD increment	No. of white roots	Y = 24.19 + (-5.43*(0.9469**X))	<.001	48.0	1.35

Appendix C

Table 3.3: Red:Green:Blue colour score values used to categorise needle colours.

Colour Description	Leaf Colour score	RGB values			Leaf Colour Percentage		
		Red	Green	Blue	% Red	% Green	% Blue
Dark Green	1	61	123	22	11	60	11
	2	83	140	37	32	54	14
	3	104	152	53	34	49	17
	4	117	167	46	35	51	14
	5	121	179	33	36	54	10
Mid-green	6	134	192	45	36	52	12
	7	143	203	29	38	54	8
	8	150	206	53	37	50	13
	9	165	215	56	38	49	13
	10	176	224	50	39	50	11
Light green	11	187	222	66	39	47	14
	12	201	232	66	40	46	13
	13	212	237	81	40	45	15
	14	217	227	92	40	42	17
	15	227	238	107	40	42	19
Pale green/ yellow-brown	16	230	243	129	38	40	21
	17	235	239	145	38	39	23
	18	235	236	158	37	38	25
	19	233	232	167	37	37	26
	20	243	224	148	40	36	24

Appendix D

Table 3.4: Root plug colonisation key used to differentiate between root plug scores.

<table>
<tr><th colspan="5">Description of Root plug</th><th>Root plug Score</th></tr>
<tr><td rowspan="5">medium falls off of plug when extracted</td><td colspan="4">no roots visible</td><td>1</td></tr>
<tr><td rowspan="4">roots visible</td><td rowspan="2">roots do not hold medium at all</td><td colspan="2">very few roots visible</td><td>2</td></tr>
<tr><td colspan="2">few roots visible</td><td>3</td></tr>
<tr><td rowspan="2">roots hold some medium</td><td colspan="2">roots present but not throughout plug</td><td>4</td></tr>
<tr><td colspan="2">roots throughout plug</td><td>5</td></tr>
<tr><td rowspan="5">little medium falls off of plug when extracted</td><td rowspan="2">no dense matting of fine roots, very soft plug</td><td colspan="3">mostly white roots</td><td>6</td></tr>
<tr><td colspan="3">brown and white roots</td><td>7</td></tr>
<tr><td rowspan="3">matting of fine roots, soft plug</td><td colspan="3">matting confined to small area</td><td>8</td></tr>
<tr><td rowspan="2">matting throughout plug</td><td colspan="2">plug sparsely colonised</td><td>9</td></tr>
<tr><td colspan="2">plug colonised</td><td>10</td></tr>
<tr><td rowspan="10">no medium falls off of plug when extracted</td><td rowspan="5">root plug firm but not hard, plug well colonised</td><td rowspan="3">low proportion of brown roots</td><td rowspan="2">lightly colonised plug</td><td>medium falls off easily if squeezed or dropped</td><td>11</td></tr>
<tr><td>medium falls off if squeezed or dropped</td><td>12</td></tr>
<tr><td>well colonised plug</td><td>medium doesn't fall off easily</td><td>13</td></tr>
<tr><td rowspan="2">high proportion of brown roots</td><td>well colonised plug</td><td>medium doesn't fall off easily</td><td>14</td></tr>
<tr><td>dense colonisation of plug</td><td>medium doesn't fall off</td><td>15</td></tr>
<tr><td rowspan="5">root plug hard</td><td rowspan="3">very high proportion of thin brown roots</td><td colspan="2">very high proportion of thin brown roots with some white root tips</td><td>16</td></tr>
<tr><td colspan="2">very high proportion of thin brown roots with no white root tips</td><td>17</td></tr>
<tr><td colspan="2">very high proportion of thin brown roots with some thick brown roots</td><td>18</td></tr>
<tr><td colspan="3">extremely dense fine brown roots with thick brown roots</td><td>19</td></tr>
<tr><td colspan="3">mainly thick brown roots</td><td>20</td></tr>
</table>

CHAPTER 4

GENERAL OVERVIEW, CONCLUSIONS AND RECOMMENDATIONS

General Overview

The threat to the *Pinus patula* forestry industry in Southern Africa from the pine pathogen *Fusarium circinatum* can potentially be overcome by family selection and hybridisation. In this regard, the taxa of most interest to Sappi Forests as a hybrid partner, identified through breeding, is *P. patula* x *P. tecunumanii* (LE). Production of hybrid seed through controlled pollination has, as expected, proved to be a limiting factor in the commercial deployment of this material as seedlings. It was therefore necessary to investigate the potential of this and other hybrid crosses using different vegetative propagation systems, that have shown to be successful in other pine taxa (Bayley and Blakeway, 2002; Cameron and Thomson, 1969; Cameron, 1968; de Assis *et al.*, 2004; Fielding, 1970; Lewis *et al.*, 1993; Majada *et al.*, 2010; Mcnabb *et al.*, 2002; Menzies *et al.*, 2001, 1986; Ritchie, 1991; Talbert *et al.*, 1993; Trueman, 2006). It was also necessary to be able to make recommendations to nurserymen on what ranges of plant morphology constituted good plant quality, in order to ensure maximum survival and growth potential of the cuttings established in field. While morphological plant quality measures have been researched, debated and successfully used for some time, in the USA especially, these have been developed for unrelated species, deployed as seedlings and many from bare-root production systems and not containerised systems (Carlson, 1986; Davis and Jacobs, 2005; Frampton *et al.*, 2002; Grossnickle and Folk, 1993; Grossnickle, 2012, 2005; Grossnickle *et al.*, 1991, 1988; Haase, 2011, 2008, 2007; Hainds and Barnett, 2006a, 2006b; Johnson and Cline, 1991; Landis and Dumroese, 2007; Marx and Hatchell, 1986; Mitchell *et al.*, 1988; Pinto *et al.*, 2011a, 2011c; Ritchie and Landis, 2010; Ritchie and Street, 2000; Ritchie, 1984; Shiver *et al.*, 1990; South and Mitchell, 2006; South and Rakestraw, 2001; South *et al.*, 2005; Sutton, 1979). However, no literature on the quality specifications of *P. patula* hybrid rooted cuttings exists, making this a completely novel area of study.

The main objective of this study was to determine the feasibility of propagating *P. patula* hybrids through rooted cuttings and to determine the required morphological specifications for *P. patula* x *P. tecunumanii* (LE) cuttings to ensure successful field establishment and growth. This chapter presents and overview of the studies undertaken.

Testing the vegetative propagation potential of *P. patula* hybrids in two possible systems.

The first specific objective of this study was to test the potential of various *P. patula* hybrids in two types of vegetative propagation systems: the existing vegetative propagation systems in use for *P. elliottii* x *P. caribaea* var. *hondurensis* hybrid cuttings at the Sappi commercial pine cuttings nursery; a new hydroponic 'sand bed' system being developed for a new cuttings nursery. The first question was whether *P. patula* hybrids could be propagated through vegetative means. This study found that *P. patula* and its hybrids could all be successfully produced through either propagation system. *Pinus patula* x *P. tecunumanii* (LE) showed the highest rooted cutting production per established hedge, due to its excellent rooting and shoot production ability. Variation in rooting and shoot production among families of this hybrid also indicated that this can be further improved through family selection.

The second question was which propagation system would be the most productive means of producing *P. patula* hybrid cuttings? The adoption of a hydroponic hedge maintenance system provided a significant increase in hedge productivity and could potentially be used to further improve the vegetative propagation of all pine taxa under investigation. The advantage of the sand bed system was better control of fertiliser application and more consistent watering. It is, however, possible that through better irrigation and fertiliser regimes the productivity of hedges grown in composted pine bark in polythene bags could be significantly improved. The use of controlled release fertilisers and irrigation systems with water sensors would be highly advantageous but will require additional resources and will result in increased production costs. This would still, however, remain a cheaper alternative to the sand beds.

A further advantage of the sand bed system is increased hedges per square meter, where space is a limiting factor. By increasing the number of hedges per square meter without decreasing productivity, and in this case actually increasing it, the space requirements to meet the nurseries production targets are significantly reduced. There would thus be less expenditure on tunnels and their associated infrastructure. The cost of producing and setting up the beds within the tunnel structure, however, is costly and would result in the overall setup costs still being higher. This said, it is also important to consider that requiring less area for hedge establishment would make complete environmental control, through completely in enclosed, temperature and moisture regulated tunnels, a much more viable financial option. This would provide a great advantage in managing of hedge productivity.

The third question investigated was whether the *P. patula* hybrids, selected for tolerance to *F. circinatum*, show improved survival over the pure species in the propagation systems. Hedge mortality due to *F. circinatum* in *P. elliottii* x *P. caribaea* var. *hondurensis* and *P. patula* x *P. tecunumanii* (LE) were significantly lower than in all other taxa. Mortality due to *F. circinatum* among open and control-pollinated *P. patula* and its hybrids with *P. tecunumanii* (HE), *P. greggii* var. *australis, P. greggii* var. *greggii* and *P. oocarpa* was high and was not significantly different to each other. Due to contamination of some crosses with *P. patula* pollen (revealed through DNA fingerprinting), it was not possible to make definite conclusions on *F. circinatum* tolerance regarding all possible hybrids. *Pinus patula* x *P. tecunumanii* (LE), however, showed low contamination and thus comparisions with the pure species could be made with this hybrid. The forth question was whether the propagation system influenced survival differences in pure *P. patula* and *P. patula* hybrids. In this trial, propagation system had no effect on the frequency of *F. circinatum* related hedge mortality.

The final question for this specific objective was if there was potential to select within *P. patula* and *P. patula* hybrid families to improve survival and rooted cutting production of hedges? This study further demonstrated that *P. patula* hedge survival could be significantly improved by identifying and selecting families that are more tolerant to *F. circinatum*. Improvement in survival, over that of the pure species families, was possible by hybridising *P. patula* with *P. tecunumanii* (LE). There were, however, no further improvements in tolerance through the selection of specific hybrid families within this cross as all showed high tolerance. The number of rooted cuttings produced per hedge was significantly higher in *P. patula* x *P. tecunumanii* (LE) than in all other taxa, especially *P. patula*. There was considerable family variation in rooted cutting production in both *P. patula* and *P. patula x P. tecunumanii* (LE). This shows the possibility of increased productivity through family selection in both *P. patula* and the *P. patula x P. tecunumanii* (LE) hybrid. Family selection, however, is ultimately based on field performance and not nursery productivity. As such, a small number of very poor performing families may be excluded from propagation as opposed to the selection of the top families only.

Testing the effect of morphological plant quality measures on field survival and growth.

The second specific objective was to quantify the effect of various morphological plant quality measures on field establishment success, in terms of survival and growth, in *P. patula* x *P. tecunumanii* (LE) rooted cuttings.

The first question for this specific objective was whether the morphological parameters identified as potentially important, that is cutting age, height, RCD, leaf colour, ectomycorrhizae presence and growing white root tip prevalence had a significant effect on cutting survival in field. All of the above phenotypic measures were found to have a significant effect on cutting survival at twelve months after planting. The largest differences in survival, in order of magnitude, were observed in needle colour, root plug score and cutting height.

Leading on from this, the second question was what the optimal ranges for those morphological parameters were. Survival in *P. patula* x *P. tecunumanii* (LE) was significantly better in dark green cuttings. Optimal survival was reached when cuttings were between eight and ten months of age; RCD of 4.0– 4.5mm; height of 28-30cm was attained and where three or more actively growing root tips were observed on the outside of the plug. Root plugs required sufficient root mass to retain some growing media in order to maximise survival.

Impact of plant morphology on field growth

Importantly, the third question aimed to establish if the phenotypic measures (cutting age, height, RCD, leaf colour, ectomycorrhizae presence and growing white root tip prevalence) also had a significant effect on cutting growth in field. Most morphological parameters showed significant effects on height and RCD increment twelve months after establishment. There were, however, exceptions. In this study the age of cuttings did not significantly affect cutting height increment. The level of ectomycorrhizae prevalence did not significantly affect the height or RCD increment of cuttings either, although its presence versus absence did. The largest differences in height growth were observed in initial height, RCD and needle colour. Initial height, RCD, root plug score and leaf colour all showed large effects on RCD increment.

Following on from this the forth question aimed to establish the optimal ranges for those morphological parameters to maximise cutting growth. Plant age did not significantly affect height growth but did impact on RCD growth. After ten months of age no further improvement was made in RCD growth. Optimal growth required dark green cuttings. Initial cutting height significantly affected height and RCD growth. Maximum height increment was observed in initial heights of 28 - 30cm and maximum RCD growth at 32 - 34cm. Initial cutting RCD significantly affected height and RCD growth. The maximum height increment was observed at an initial diameter of 3.5mm and maximum RCD growth between 4.0 – 4.5mm. Optimum height increment was reached by a plug score of 7, after which no further change was observed. Optimum RCD increment was only reached at a root plug score of 14. In this category, no media fell off of plug when extracted; the root plug was firm but not hard and was well colonised with a high proportion of thin brown roots. While three or more actively growing white root tips are sufficient to optimise survival, there were growth gains from having more.

While plant growth at one year was used to determine the ideal morphological specification range of the cuttings at planting it is important to note that these differences may not continue to rotation age as the limitations of the container environment are eclipsed by the effects of the field conditions and plant genetics (Pinto *et al.*, 2011b). The one year growth results from this study can thus only be used to hypothesise where possible growth differences may manifest, but in order to make accurate predictions of the effects of *P. patula* x *P. tecunumanii* (LE) cutting morphology on long term growth, further measurements are required throughout the rotation.

Application of cutting morphology to optimize field survival and growth

With multiple ranges of possible morphological measures to maximise survival, height growth and RCD growth, question five aimed to consolidate these observations into combined ideal recommendations for cutting age, height, root collar diameter, leaf colour, ectomycorrhizae presence and growing white root tip prevalence. The ideal raising period for *P. patula* x *P. tecunumanii* (LE) cuttings is 10 months from setting as: optimal survival was reached at ages between eight and ten months; there was no age effect on height increment and there was no increase in RCD growth after ten months of age. Any additional time in the nursery would not increase field growth or survival potential. Longer periods in the nursery would, however, increase costs through increased nursery inputs per cutting produced and limit production capacity through increased occupancy of cuttings in the nursery. Within Sappi Forests the current ideal age recommendation for *P. elliottii* x *P. caribaea* var. *hondurensis* and *Pinus patula* hybrid cuttings, based on early studies and nursery experience, is 8 - 10 months. The findings of this study thus show that this is an appropriate age allocation for all three taxa, although eight months may be too young.

For initial cutting height, optimal survival was reached at 28 - 30cm, maximum height increment between 28 - 30cm and maximum RCD growth at 32 - 34cm. The ideal height for *P. patula* x *P. tecunumanii* (LE) cuttings, in good planting conditions, was thus 28 - 32cm. The current height recommendation for *P. elliottii* x *P. caribaea* var. *hondurensis* and *P. patula* hybrid cuttings is 7 – 20 cm with 11 - 15cm being recorded as ideal. The results of this trial show that these recommendations are not suitable for *P. patula* x *P. tecunumanii* (LE) cuttings. It also seems to suggest that the ranges for *P. elliottii* x *P. caribaea* var. *hondurensis* and *Pinus patula* hybrid cuttings may require revision. This finding is likely to result in increased establishment success of all pine rooted cuttings within Sappi Forests.

Cutting RCD showed optimal survival at 4.0 – 4.5mm with maximum height increment at 3.5mm and maximum RCD growth between 4.0 – 4.5mm. The ideal RCD range for *P. patula* x *P. tecunumanii* (LE) cuttings was thus 3.5 - 4.5mm. The current RCD recommendation for *P. elliottii* x *P. caribaea* var. *hondurensis* and *Pinus patula* hybrid cuttings is 2.5 – 3.5mm with 2.9 – 3.2mm being ideal. The findings of this trial suggest that these recommendations for the current pine hybrids would not be suitable for *Pinus patula* x *Pinus tecunumanii* (LE) cuttings, which require slightly broader root collars in order to survive optimally. This finding questions the current recommendations and suggests that these may also need to be revised.

The colour of cuttings, and the nutrient status this infers, was of great importance to both survival and growth in *P. patula* x *P. tecunumanii* (LE) cuttings. An important outcome of this study was the development of novel categorisation and scoring systems for plant colour and root plug colonisation (Appendix E – I). The twenty category colour scale, with decreasing intensity of greenness, was highly effective in categorising plants. This statement is supported by the excellent regression relationships observed in the effect of colour on survival, height growth and RCD growth. The success of this system lies in having photographed varying degrees of greenness displayed by the cuttings, selecting dominant colour from the image and using both the picture and colour block in a colour chart visual aid for the team scoring the cuttings. The existing scoring scale consisted of a subjective three category scale of 'Deep green', 'Yellow/Green' and 'Yellow/ Brown' only, which would not have allowed for the quantitative measures required for regression analysis, as completed in this study. To optimise both survival and growth in these cuttings required dark green cuttings scoring between 1 and 8 on the colour scale. Both height and RCD growth showed linear increases with increasing greenness which suggests that the greener cuttings the better. The current colour recommendation for *P. elliottii* x *P. caribaea* var. *hondurensis* and *Pinus patula* hybrid cuttings is that they are deep green. The findings of this study confirm that this is the correct recommendation. The current recommendation does, however, have yellow/green colour as acceptable. The results of this trial clearly show that there are rapid decreases in survival and growth in this colour range and the recommendation should therefore be adjusted to allow for only dark greens.

The root plug colonisation scale was also developed with the use of photographs. Photographs of increasing plug colonisation were grouped into twenty categories from least colonised to most moribund (Appendix E – II). In addition to this, a descriptive partial dichotomous key was developed to be used in conjunction with the photographs as a combined aid for teams scoring the root plugs. The existing scoring scale consisted of a subjective four category scale of 'Not colonised', 'Partially colonised', 'Colonised, not root bound' and 'Root bound', which would not have allowed for the quantitative measures required for regression analysis, as completed in this study.

Even under ideal planting conditions plugs required sufficient root mass to retain some growing media in order to survive which was represented by root plug scores of 3 or above. With optimal height increment at plug scores of 7 and above and optimum RCD increment at plug scores of 14 or above, a plug score of 14 represented the minimum requirement to maximise all three parameters. The recommendation from this study would thus be to ideally dispatch plants in category 14 where no media falls off of plug when extracted, the root plug is firm but not hard and the plug is well colonised with a high proportion of thin brown roots. Scores above 14 would increase the risks associated with being root bound and would decrease nursery productivity through longer occupancy of bed space. These findings support the current recommendation which is to dispatch cuttings which have fully colonised root plugs, without being root bound. Although partially colonised root plugs are currently acceptable, albeit less acceptable, this study has shown that in the bottom range of this category there may be reduced growth in the first year. The effectiveness of the scale used in this study is difficult to ascertain as excellent planting conditions meant that only the poorest two categories showed a significant impact on survival. Under greater water stress and poorer planting practice we would expect to see greater differences between those plants with poorly developed root systems and those which were well colonised. The effects of being increasingly root bound would also not have manifested in survival differences at one year, but might at two years of age or older. The results of this study did suggest decreased growth with increasingly moribund root plugs but this can only be confirmed with further measurements throughout the rotation. Further work on the root plug categorisation scale is therefore required to provide more conclusive evidence of its efficacy.

The presence of actively growing root tips on the outside of the plug was important in the survival and growth of *P. patula* x *P. tecunumanii* (LE) cuttings with three or more tips to optimise survival and growth gains achieved by having more. The current recommendation is a subjective measure which estimates the proportion of the root plug which has white root tips, and is grouped as <25%, 25 – 50 % and >50%. It is suggested that < 25% is not acceptable, 25 – 50 % is ideal and > 50 % is acceptable but not ideal. Grouping the number of white root tips with such a subjective measure is problematic as its reliability would be questionable at best. While having root tips present in more than 25% of the root plug would certainly be advantageous, it's estimation by varying observers would tend to differ and lack repeatability. The recommendation would therefore to remove this as a measure and replace it with a quantitative measure as reported in this trial. A further problem with the existing system is that is suggests that too many white root tips are not ideal. This study shows that there is no survival disadvantage to having a high proportion of white root tips, and that there is in fact growth advantages to this condition. A maximum number of white roots is therefore not required.

It is also essential to consider that plant quality recommendations provide a predicted estimate of a plant's ability to survive and grow under normal planting conditions and is not a guarantee of survival under all conditions (Grossnickle, 2012). Extended holding periods in field, adverse planting conditions and poor planting practice would compromise these predictions.

Conclusions and recommendations

This study confirms that a number of *P. patula* hybrids can successfully be produced by means of rooted cuttings using either composted pine bark growing media in polythene bags or in hydroponic sand beds. The required morphological specifications for *P. patula* x *P. tecunumanii* (LE) cuttings, to ensure successful field establishment and growth under good growing conditions, was also established. Further work to validate and refine these specifications and to potentially generate a model accounting for the relative importance of each parameter would be of great value to the industry.

Recommendations

The results of this study showed that through increased survival and productivity, the *P. patula x P. tecunumanii* (LE) hybrid was better suited to vegetative mini-hedge propagation systems than *P. patula*, which had previously been attempted commercially and was not successful. The *P. patula x P. tecunumanii* (LE) hybrid was also highly productive, being comparable to or better than the current commercial taxon *P. elliottii* x *P. caribaea* var. *hondurensis.* The *P. patula x P. tecunumanii* (LE) hybrid can thus be successfully deployed as rooted cuttings in a commercial nursery.

The hydroponic sand bed system, as pioneered in Brazil (de Assis *et al.*, 2004) would be the recommended production method for producing the *P. patula x P. tecunumanii* (LE) hybrid. The hydroponic system provides superior fertigation, irrigation and productivity. Hedges should be established at an espacement of 15 x

10cm with fertigation applied at a concentration between 1200 and 1500 µS/cm, once daily for 20 minutes in the morning via drip lines (pressure compensated, non-leaking, delivering 1.1L per hour per dripper). Beds should be flushed with pure water every one to two weeks, depending on the EC of the leachate. With close control of watering regimes and the use of controlled release fertilisers, however, the composted pine bark in polythene bags propagation method remains a viable option, albeit less productive, where seed availability for hedge establishment and nursery space are not limiting factors and capital costs might be.

The *P. patula x P. tecunumanii* (LE) hybrid was shown to be highly tolerant of *F. circinatum*, and therefore, offers a viable alternative to *P. patula* in a vegetative propagation system. There was also evidence that family selection within *P. patula* could be used as an interim step to improve hedge survival in commercial nurseries while sufficient stocks of the hybrid seed are generated. The survival and productivity of alternate *P. patula* hybrid combinations to *P. patula x P. tecunumanii* (LE) requires further investigation due to the observed *P. patula* x *P. patula* contamination. It would therefore be recommended that subsamples of *P. patula x P. tecunumanii* (LE) hybrid seed batches for commercial hedge establishment, be sampled using DNA fingerprinting to estimate the purity of the batches before establishment and production.

The one year field survival of rooted cuttings provided a good estimate of potential establishment success of *P. patula* x *P. tecunumanii* (LE) cuttings under good planting conditions. This was supported by morphological parameters, which showed peak survival above 90%. These findings can thus be used to predict optimal survival under these conditions. Recommendations for ideal cutting morphology were generated in this study (Table 1). Based on these data the prime raising period for *Pinus patula* x *Pinus tecunumanii* (LE) cuttings, grown in a 90 ml cavity under the nursery conditions described in Chapter 3, is 10 months from setting. The ideal height for these cuttings was 28 - 32cm and the ideal RCD range was 3.5 - 4.5mm. The root plug integrity recommendation from this study is to dispatch plants with a score of 14, with at least three or more actively growing white root tips and visible evidence of ectomycorrhizae. Needles must be in the dark mid-green to dark green range scoring 8 or better on the scale.

Table 4.1: Ideal plant quality specifications for *P. patula x P. tecunumanii* (LE) rooted cuttings.

		Required Level		
Quality Category	**Quality Measure**	**Minimum**	**Ideal**	**Maximum**
Age	Age (Months)	8	10	11
Size	Height (cm)	28	30	32
	Root Collar Diameter (mm)	3.5	4.0	4.5
Health	Needle colour score		1-8	
Root Development	Root Plug score	11	14	15
	No. of white Root Tips	3	>3	
	Ectomycorrhizae	present		

This study highlighted that the current cutting plant quality recommendations within Sappi for pines, in their entirety, would not be suitable for *Pinus patula* x *Pinus tecunumanii* (LE). These early recommendations for *P. elliottii* x *P. caribaea* var. *hondurensis* and *Pinus patula* were based on very little trial data, where all parameters were not simultaneously tested. It was also extrapolated, to some extent, from international seedling research and using subjective estimation by Sappi nurserymen.

It was reassuring to see that the recommendations for plant age, root plug colonisation and needle colour would not need to be greatly changed for the three taxa combined. The requirements for plant height and RCD, however, were substantially different to the current pine cutting recommendations and hence require attention. It is probable that the specifications for height and RCD should be adjusted up but at this juncture it may not be wise to adjust them up as far as the findings of this study, listed above, alone. The reason for this is that this single trial accounts for only non-adverse sites. Various studies have shown that survival in seedlings increases with increasing height on non-adverse sites or where weed competition is prevalent (Mohammed *et al*. 1998; South and Mitchell 1999; Puértolas *et al*. 2003) while on adverse sites, survival decreases with height (Larsen et al. 1986; Boyer and South 1987; Tuttle et al. 1988; McTague and Tinus 1996). The *Pinus patula* x *Pinus tecunumanii* (LE) cuttings would thus require testing on adverse sites as well, to fully understand the relationship between plant size and survival.

Future studies

A limitation of this study is that it was carried out on a single site under good planting conditions and hence does not reflect all possible interactions with varying site quality and planting conditions. The recommendations made from these data should therefore be seen as a solid starting point that requires further validation across multiple sites, and under moisture stress conditions, in order to provide greater predictive accuracy.

Although this study has identified important plant quality specifications for *P. patula* x *P. tecunumanii* (LE) rooted cuttings, it does not account for the relative importance of each morphological parameter in determining establishment success. Neither does it consider the possible co-linearity of parameters and their independent effect on survival and growth. An example of this is the highly correlated effects of age, height and RCD on survival and growth. Estimating the relative importance of each parameter independently of each other would require analysis accounting for the multi-co-linearity. This analysis could also potentially allow a more parsimonious plant quality model to be developed, with less parameters requiring measurement.

The inclusion of further sites to refine quality recommendations for *P. patula* x *P. tecunumanii* (LE) rooted cuttings and the development of a novel and parsimonious model, accounting for the relative importance of individual morphological parameters, requires further study.

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Appendix E

I) LEAF COLOUR VISUAL AID

II) ROOT PLUG INTEGRITY VISUAL AID

1	2	3	4	5	6	7	8	9	10
media falls off of plug when extracted					little media falls off of plug when extracted				
no roots visible	roots visible				no dense matting of fine roots, very soft plug		matting of fine roots, soft plug		
	roots do not hold media at all		roots hold some media		mostly white roots	brown and white roots	matting confined to small area	matting throughout plug	
	very few roots visible	few roots visible	roots present but not throughout plug	roots throughout plug				Plug sparsly colonised	Plug colonised
1	2	3	4	5	6	7	8	9	10

11	12	13	14	15	16	17	18	19	20
no media falls off of plug when extracted					no media falls off of plug when extracted				
root plug firm but not hard, plug well colonised					root plug hard				
Low proportion of brown roots			High proportion of brown roots		Very high proportion of thin brown roots			exremely dense fine brown roots with thick brown roots	mainly thick brown roots
lightly colonised plug		Well colonised plug	Well colonised plug	Dense colonisation of plug	very high proportion of thin brown roots with some white root tips	very high proportion of thin brown roots with no white root tips	very high proportion of thin brown roots with some thick brown roots		
Media falls off easily if squeezed or dropped	Media falls off if squeezed or dropped	Media doesn't fall off easily	Media doesn't fall off easily	Media doesn't fall off					
11	12	13	14	15	16	17	18	19	20