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

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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.

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

Table 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.

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 applie d 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, via six lines of Netaf im 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. 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*.

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

Table 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
Total	2452	1111	22

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.

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.

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)

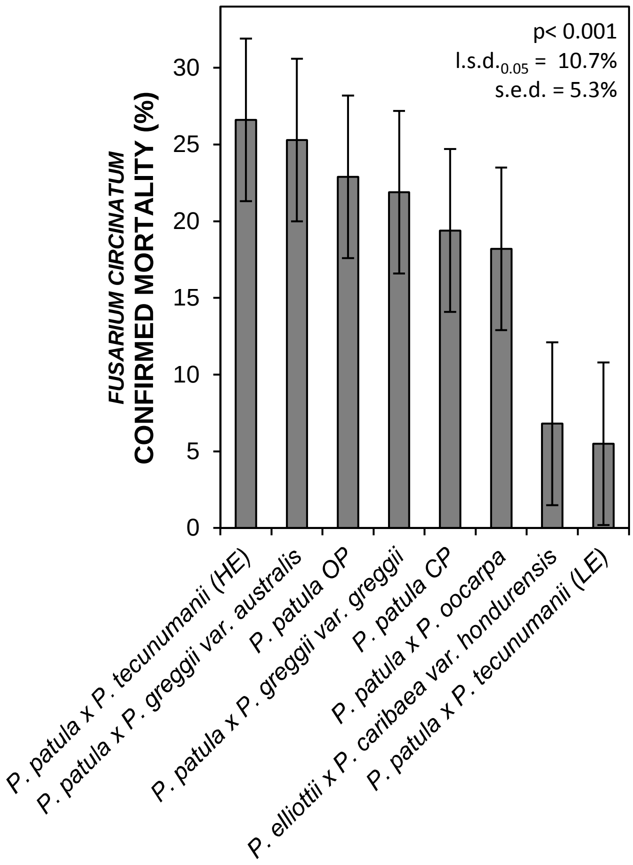

Figure 1: Hedge stock plant mortality per taxon that was positively linked to infection with *F. circinatum* in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. OP = open-pollinated; CP = controlled-pollinated; HE = High elevation; LE = Low elevation. Error bars represent the standard errors of differences of means.

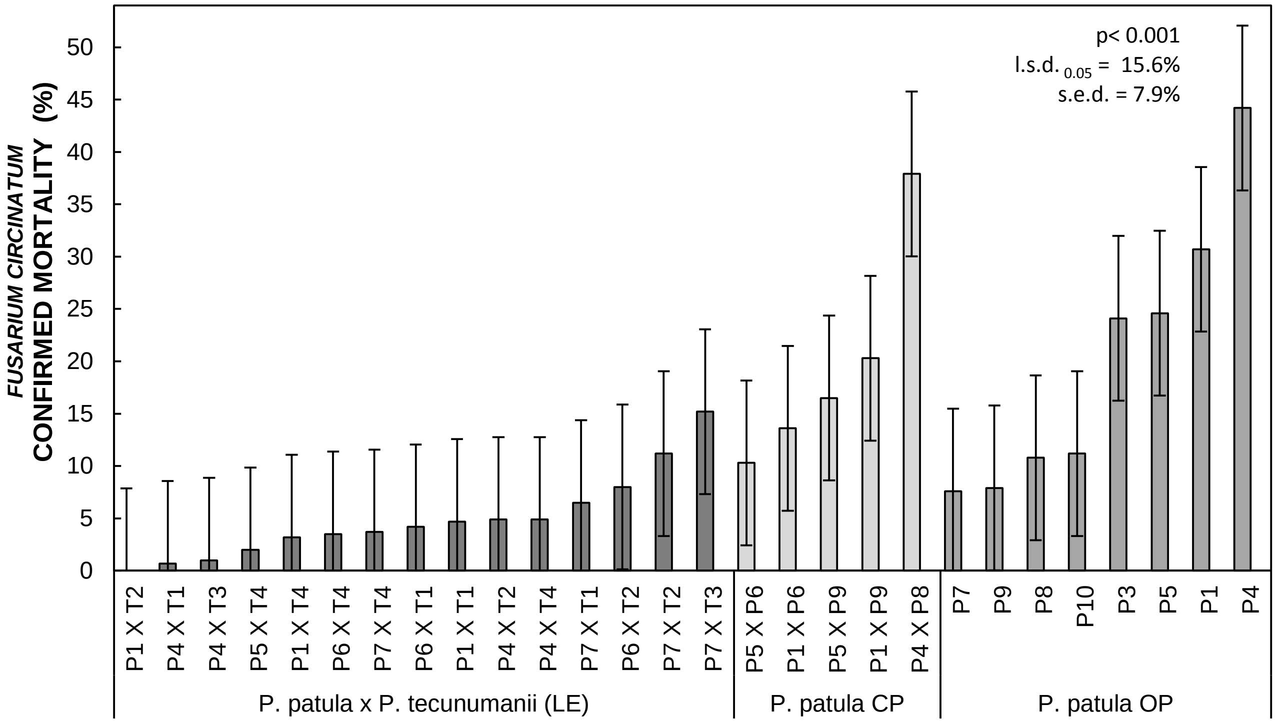

Figure 2: Hedge stock plant mortality for *P. patula* (controlled-pollinated and open pollinated) and *P. patula* x *P. tecunumanii* (LE) families that were positively linked to infection with *F. circinatum* in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. P = *P. patula* parent, T = *P. tecunumanii* parent. Error bars represent the standard errors of differences of means.

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). 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*.

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). There was no significant difference in

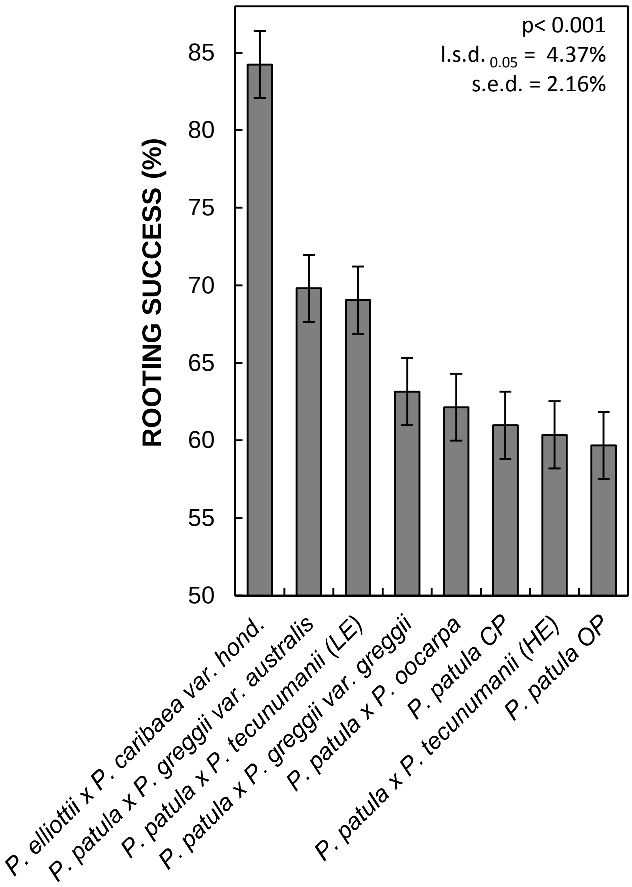

Figure 3: Mean rooting percentage of cuttings from pure and hybrid taxa over a 45 month period (20 settings) in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. Error bars represent the standard errors of differences of means.

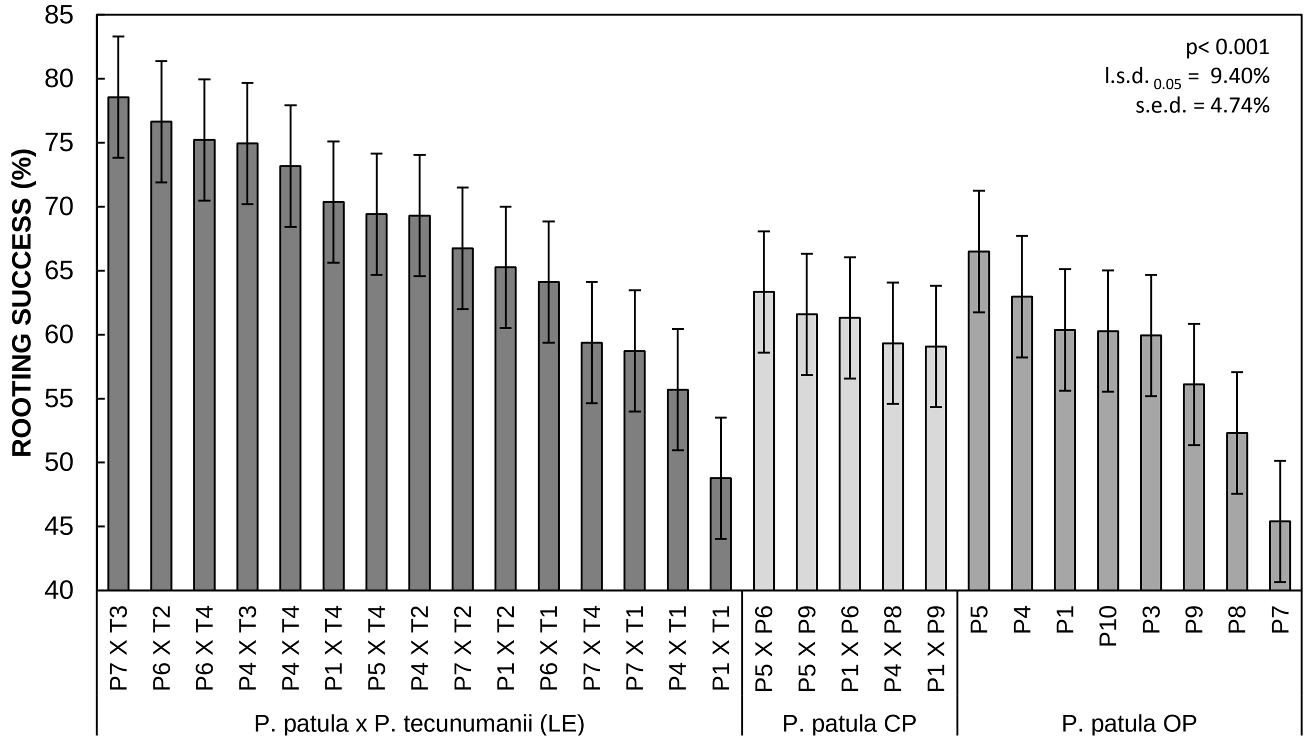

Figure 4: Mean rooting percentage of cuttings from *P. patula* (control-pollinated and open-pollinated) and *P. patula* x *P. tecunumanii* (LE) families in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. P = *P. patula* parent, T = *P. tecunumanii* parent. Error bars represent the standard errors of differences of means.

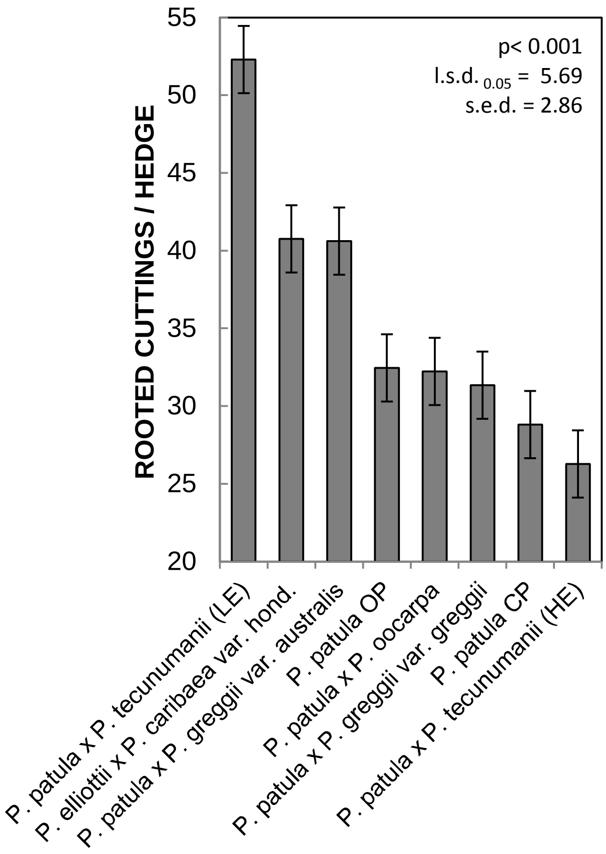

Figure 5: Number of rooted cuttings established per hedge from the pure and hybrid taxa over a 45 month period (20 settings) in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. Error bars represent the standard errors of differences of means.

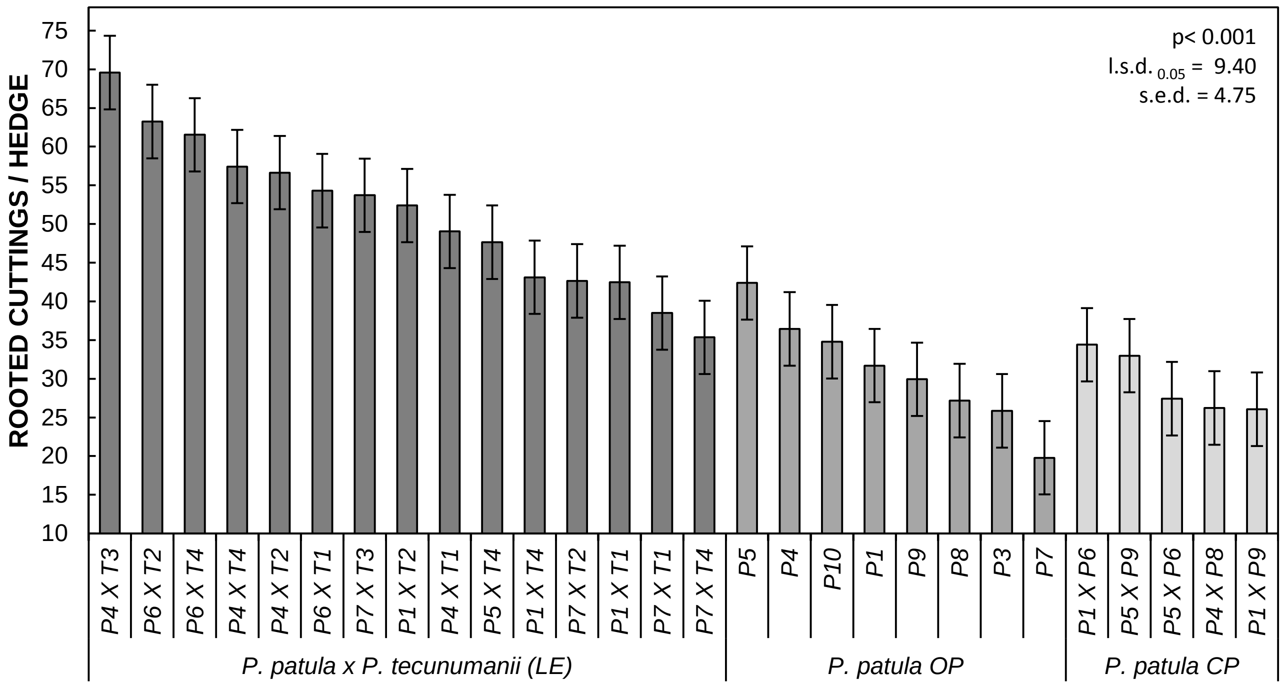

Figure 6: Rooted cutting production per family for *P. patula* (control-pollinated and open-pollinated) and *P. patula* x *P. tecunumanii* (LE) in bagged hedges in two experiments testing *P. patula* and *P. patula* hybrid vegetative propagation potential and *F. circinatum* tolerance. P = *P. patula* parent, T = *P. tecunumanii* parent. Error bars represent the standard errors of differences of means.

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.

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). There was no interaction between propagation system and species (p = 0.664), all

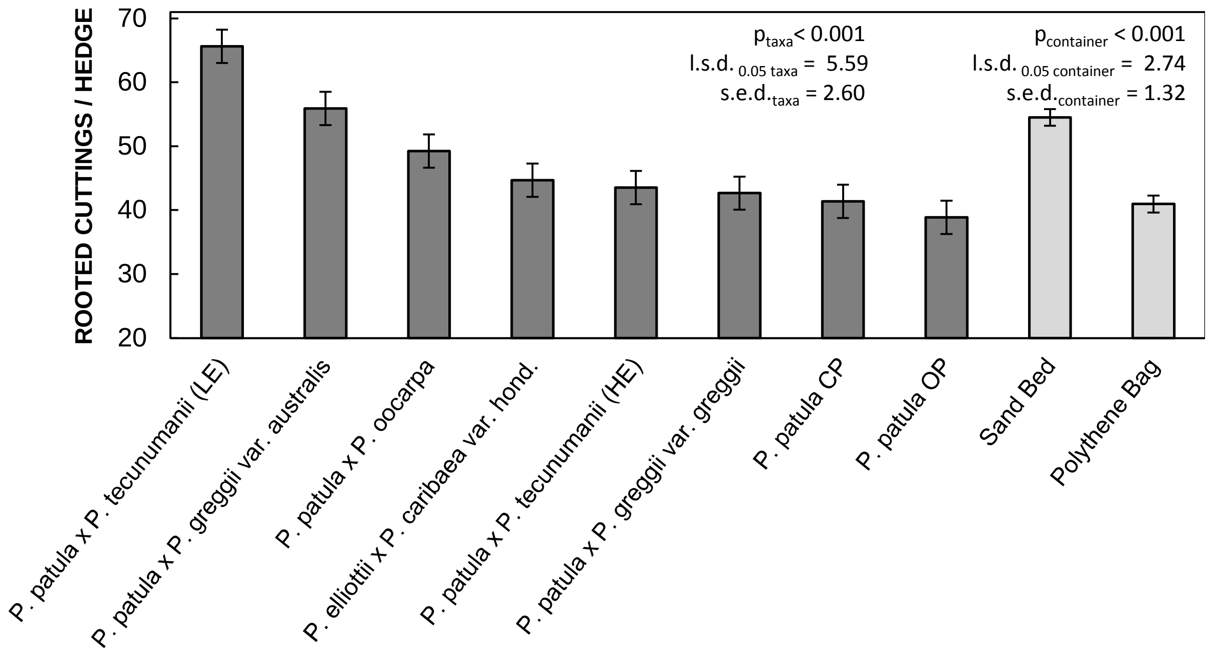

Figure 7: Number of rooted cuttings established per hedge from the pure and hybrid taxa and two propagation systems, in experiment 2, over a 45 month period (20 settings). Error bars represent the standard errors of differences of means.

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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