

Development and commercialisation of the *Pinus patula* × *P. tecunumanii* hybrid in response to the threat of *Fusarium circinatum*

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Abstract During the last 20 years a program to develop and commercialize the *Pinus patula* × *Pinus tecunumanii* hybrid, as a replacement for *P. patula*, has been successfully implemented. The first crosses were initiated during the early 1990s and lead to establishment of field trials across a wide variety of sites. This work gained further impetus when it became apparent that *Fusarium circinatum*, was causing poor post-planting survival of newly established stands of *P. patula*. *P. tecunumanii*, has been shown to be tolerant to this disease and thus a second, more comprehensive hybrid testing phase was implemented. Improvements in controlled pollination techniques and propagation methods, as well as access to genetically improved parent-stock and the use of molecular marker technology for fingerprinting was effectively utilized to greatly improve the process during this second phase. The use of artificial inoculation trials have demonstrated that the hybrid, in particular when using the low elevation (LE) provenances of *P. tecunumanii*, had substantially greater tolerance to *F. circinatum* than *P. patula* and survival figures from field trials support these results. Four-year volumes also indicate large growth improvements, although frost damage on certain sites presents a challenge for deployment on colder sites; and this is being tackled through breeding and accurate mapping of frost risk. Large-scale controlled pollinations and vegetative multiplication are now utilized commercially to produce the *P. patula* × *P. tecunumanii* (LE) hybrid as an alternative to *P. patula*.

Keywords *P. patula* · *P. tecunumanii* · *Fusarium circinatum* · Disease and frost tolerance · Hybrid breeding

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Introduction

Pinus patula Schiede ex Schlecht. & Cham. is currently the single most widely distributed softwood species planted in southern Africa, and represents around half of the total area planted to softwoods (FES 2008). According to Dvorak et al. (2000a), *P. patula* requires deep, well drained soils and grows best in the mist belt regions of South Africa above 1,000 m elevation. In southern Africa the species is generally planted in areas with mean annual temperatures below 17 °C (Morris and Pallett 2000).

Pinus tecunumanii Eguiluz & J. P. Perry is a species found in Mexico and Central America and two distinct groups are recognized: (1) trees from high elevation populations that occur from approximately 1,500–2,900 m altitude in Honduras, Guatemala and Mexico and, (2) low elevation (LE) populations that occur from approximately 450–1,500 m altitude in Nicaragua, Honduras and Belize (Dvorak et al. 2000b). These two groups differ in a number of ways. The high elevation has slightly poorer tolerance to *Fusarium circinatum* (Hodge and Dvorak 2000), lower wood density (Malan 1994) and better cold tolerance (Kanzler 2007; Hodge et al. 2012). *P. tecunumanii*, as a species, offers a number of advantages over *P. patula*: these include rapid growth in the nursery, better drought tolerance, higher productivity (Hodge and Dvorak 2012), higher wood density, more uniform wood (Malan 1994; Dommissie 1994) and better tolerance to *F. circinatum* (Hodge and Dvorak 2000).

Fusarium circinatum was first identified in a South African nursery in 1990 (Viljoen et al. 1994) and was essentially restricted to nurseries until 2000. Since then the pathogen has caused severe post-establishment mortality with *P. patula* in commercial plantations (Crous 2005). *Pinus patula* has been identified as being particularly susceptible to the pitch canker fungus (Hodge and Dvorak 2000), and indeed, *F. circinatum* poses a serious threat to the continued use of pure *P. patula* as a commercial species (Morris 2010; Mitchell et al. 2012a). Since then the disease has been found in older trees in plantations of *P. radiata* in the moist areas in the southern Cape coastal zone (Coutinho et al. 2007).

Inter-specific hybrids provide the potential to combine the advantages of both parent species and have been used successfully in tree species since the early to mid-1900s (Dungey 2001). Almost 30 years ago (Barnes and Styles 1983) suggested that there may be great potential in crossing *P. patula* with some of the other closed-cone pines to produce hybrids that could be better adapted than the pure species to the environmental conditions that exist in southern Africa. *Pinus patula* belongs to the *Oocarpae* subsection that includes other Mesoamerican closed-cone pines *P. oocarpa*, *P. tecunumanii* and *P. greggii* (Dvorak et al. 2000c; Price et al. 1998). These species have been imported into South Africa over a number of years, and comprehensive breeding populations have been established. South African breeders therefore now have an excellent opportunity to manipulate and utilize this variation and make substantial gains through hybridization with *P. patula* (Dvorak 2000).

This paper reviews work done over 20 years in developing the *P. patula* × *P. tecunumanii* hybrid as an example of a case study of pine hybrid commercialisation. The focus has been on work done within the author's company (Sappi) but several other companies in South Africa have had to deal with similar challenges and used similar strategies to overcome these problems. The project documents the initial wide scale field testing of the hybrid using opportunistic crosses in the 1990s: the *F. circinatum* screening of the hybrid, both in the nursery and in the field; the optimisation of propagation protocols; the field testing of improved material for operational use; the assessment of wood properties of the two parent species and the hybrid; approaches employed to deal with frost damage experienced with the hybrids on certain sites; and concludes with a discussion around the

future breeding and use of the hybrids within the context of challenges faced by breeders in southern Africa.

The selection of the *P. patula* × *P. tecunumanii* hybrid

A number of potential hybrids were considered (refer to Table 1). The selection of *P. tecunumanii* as the hybrid partner was made for a number of important reasons: high growth potential of the species, large breeding base in provenance trials and breeding banks, early availability of pollen, close genetic distance with *P. patula* (correlated with the relative ease in making the cross), high wood density and good tolerance to *Fusarium circinatum*. Other perceived advantages of the species, based on experience in establishing and growing *P. tecunumanii* in field trials over a range of sites, included rapid growth in the nursery, rapid site capture reducing prolonged weed competition and relatively good drought tolerance.

Pinus tecunumanii is a more tropical species than *P. patula*. It is found at latitudes closer to the equator, especially in the case of the LE sources, on warmer, lower sites. Thus one of the key disadvantages of *P. tecunumanii* is relatively poor cold/frost tolerance. This has been demonstrated in a number of field trials within the region (Falkenhagen 1990; Mitchell et al. 2013). A number of other potential disadvantages related to the species are

Table 1 Species identified as potential hybrids with *P. patula*, species are listed in order of increasing genetic distance from *P. patula*

Species	Genetic distances ^a	Key advantages traits associated with potential hybrid partner	Evidence of previous success as a hybrid partner with <i>P. patula</i> ^b
<i>P. greggii</i> var. <i>australis</i>	0.095	Better adapted to warmer, harsher sites than <i>P. patula</i>	Successful crosses have been made. Field trials demonstrated excellent growth and adaptability (Kanzler et al. 2012)
<i>P. greggii</i> var. <i>greggii</i>	0.095	Cold tolerant and very drought tolerant on cooler sites	No confirmed crosses
<i>P. oocarpa</i> (Mexico)	0.134	Resistant to <i>Fusarium circinatum</i> , greater wood density and easy to propagate as cuttings	Successful crosses have been made (Critchfield 1967)
<i>P. tecunumanii</i> (low elevation)	0.135	High productivity, greater wood density and good tolerance to <i>Fusarium circinatum</i>	Successful crosses have been made. Field trials demonstrated good establishment, growth and <i>Fusarium circinatum</i> tolerance (Roux et al. 2007)
<i>P. tecunumanii</i> (high elevation)	0.143	High productivity, greater wood density and moderate tolerance to <i>Fusarium circinatum</i>	Successful crosses have been made. Field trials demonstrated excellent growth and adaptability (Kanzler et al. 2012)
<i>P. oocarpa</i> (Central America)	0.175	Resistant to <i>Fusarium circinatum</i> , greater wood density and easy to propagate as cuttings	Successful crosses have been made. Field trials demonstrated good establishment, growth and <i>Fusarium circinatum</i> tolerance (Roux et al. 2007)

^a Genetic distances from *P. patula* (Dvorak et al. 2000c); ^b All of the above crosses, with the exception of *P. patula* × *P. oocarpa* (Mexico) hybrid, have been attempted as part of a CAMCORE cooperative pine hybrid project and all of these latter five, except the *P. patula* × *P. greggii* var. *greggii* hybrid, have been confirmed using molecular markers and planted out in field trials (Camcore Annual Report 2008a, b, 2009)

the tendency for stem breakage measured in unimproved, provenance trials (Dvorak et al. 1993) and the reputation of the species as a poor flowerer, on some sites, thus limiting the availability of pollen (Dvorak and Lambeth 1993).

An outline of the approach taken in development of the *P. patula* × *P. tecunumanii* hybrid

In testing any new taxon (species or in this case hybrid), a number of key issues need to be considered in order to evaluate the proposed taxa as a viable alternative. In this study the following questions were posed and addressed:

Is the hybrid well adapted for the sites where it is targeted?

Will it survive after planting?

Will it grow well?

Is the crop healthy—free of diseases and pests?

Can it withstand local climatic challenges such as drought, frost or snow?

Can the hybrid be propagated cost effectively and efficiently?

Is the hybrid ‘acceptable’ as a plantation crop?

Is the hybrid ‘acceptable’ as an industrial raw material for the market that will be supplied?

The project covered in this paper encompasses work that commenced with controlled crosses to produce the hybrid in 1991 to the present day commercialisation of the hybrid within Sappi. The work can essentially be divided into four phases that are outlined in Table 2.

Initial tests of adaptability and acceptability of the *P. patula* × *P. tecunumanii* hybrid

The primary focus for working with the *P. patula* × *P. tecunumanii* hybrid was as a replacement for *P. patula*. *P. patula* is planted in the across the full range of site qualities in the cool temperate and cooler, warm temperate climatic zones in southern Africa (Smith et al. 2005). Hybrid testing was thus focused on these sites although sites in the lower areas of the warm temperate zone were also targeted.

Table 2 Details of the four phases of the project to produce, assess and commercialise the *P. patula* × *P. tecunumanii* hybrid within Sappi

Phase	Description of the focus and emphasis within that phase	Time frames
Phase 1	Production of hybrid crosses with opportunistic use of any <i>P. tecunumanii</i> pollen available in unselected trees in provenance trials; comprehensive field testing of the hybrid to evaluate adaptability, growth and ‘acceptability’	1991–2007
Phase 2	Rapid development, early testing and deployment of a set of crosses, using low elevation <i>P. tecunumanii</i> sources from selected trees using the polymix testing strategy	2003–2012
Phase 3	Production and testing of a range of potential alternative taxa (species/hybrids) to evaluate the efficacy of the selected hybrid	2005—present
Phase 4	Production and testing of a large number of additional hybrid families using and large factorial mating design	2007—present

Methodology

*Controlled pollination (CP) success with the *P. patula* × *P. tecunumanii* hybrid*

The ability to produce seed when making the hybrid cross and the ease of making the cross are important considerations when assessing the potential of a specific pine hybrid combination. With current technology a clonal propagation route with pines remains challenging and thus, provided propagation technology is not prohibitively expensive, making the cross and multiplying the seed via cuttings currently remains the most cost effective route for commercialising pine hybrids in southern Africa. The cone and seed production capacity, when making CP crosses to produce the *P. patula* × *P. tecunumanii* hybrid, as well as published data on the capacity of the two parent species, is presented in Table 3. There is a large reduction in average seeds per cone, when making the hybrid, which may be due to the controlled pollination process or hybrid production using *P. tecunumanii* rather than *P. patula* as the pollen parent (Table 3).

Crosses made and time frames with propagation

The first crosses to produce *P. patula* × *P. tecunumanii* hybrids were made using any available pollen and crossed with *P. patula* opportunistically. The objective during this phase was to test the hybrid as rapidly and broadly as possible in order to assess the potential for this taxon. The pollen utilized during this phase was mostly unselected *P. tecunumanii* (sometimes from relatively poor-looking trees) present within provenance trials. This phase included the production of crosses with both high (from Las Piedrecitas, Napite and Pachoc provenances) and low (Culmi and San Rafael provenances) elevation sources and occurred from about 1991 to 2003. It should be noted that although an attempt was made to collect and produce as many crosses as possible to provide a representative

Table 3 A comparison of cone and seed production for the *P. patula* × *P. tecunumanii* hybrid and the two parent species

	<i>P. patula</i> (Mexico) ^a	<i>P. patula</i> (South Africa) ^b	<i>P. tecunumanii</i> (high elevation) ^c	<i>P. tecunumanii</i> (low elevation) ^c	<i>P. patula</i> × <i>P. tecunumanii</i> (LE) ^b
Cone size (mm)	55–100	70–100	30–93	40–75	NA
Nr of cones/ cluster	2–14	1–17	1–2	2–4	NA
Average seeds/ cone	125	60–80	90	95	37
Filled seeds/cone	22	64	6	30	15
Efficiency (filled seeds/average seeds × 100 (%))	18	72	7	32	15
Seeds/kg	118,000	100,000	88,000	76,000	NA

^a Dvorak et al. (2000a)

^b Solomon pers. comm. (2013)

^c Dvorak et al. (2000b)

sample of the populations, in practice this was not always possible, and some populations were represented by only a few families.

Testing and propagation strategy

The amount of seed produced was small and thus in most cases the material was propagated as cuttings harvested from seedling hedges using a similar technique developed for *P. patula* (Bayley and Blakeway 2002). This material was then planted out across twenty-five sites, broadly representing areas from both the cool and warm temperate zone. Details of sites, hybrid material and a brief field trial description are provided in Table 4.

Table 4 *P. patula* × *P. tecunumanii* hybrid field trials established as part of the initial phase of testing

Source of <i>P. tecunumanii</i>	MAT (°C)	MAP (mm)	Altitude (m)	Planted	Description of field test
Las Piedrecitas (high elevation)	16.6	866	1,365	1996	3–5 Families; trial designs ranged from unreplicated line or block plantings to randomised complete blocks with nine replications; assessment age between 4 and 8 years; all hybrids propagated as cuttings
	18.6	835	1,000	1997	
	14.8	1,247	1,600		
	14.9	1,093	1,500		
	15.8	1,124	1,240		
	16.3	1,421	1,320		
	17.1	1,350	1,060		
	15.8	1,124	1,240		
	16.3	1,421	1,320		
	16.6	858	1,300		
	14.8	775	1,700		
	17.1	1,350	1,050		
17.0	982	1,050			
Culmi (low elevation)	16.6	866	1,300	1999	3–5 Families; trial designs ranged from unreplicated line or block plantings to randomised complete blocks with six replications; assessment age between 3 and 7 years; all hybrids propagated as cuttings
	13.9	912	1,800	2001	
	18	1,350	980		
	18	874	917		
	16.8	1,350	1,097		
	16.8	1,050	1,209		
	15.8	1,124	1,280		
Napite and Pachoc (high elevation)	16.6	858	1,300	1998	8–24 Families; randomised complete blocks with 3–6 replications; assessment age 3–5 years; hybrids propagated as cuttings or seedlings
	14.8	1,247	1,626	2002	
	15.7	1,150	1,338		
San Rafael (low elevation)		900	925	Feb.'00	Two families; RCB with nine replications; Assessment age 5 years propagated as seedlings

MAT mean annual temperature (°C), MAP mean annual precipitation (mm)

Results and discussion from this first phase of testing

Establishment and survival

The establishment and survival of the *P. patula* × *P. tecunumanii* hybrid was variable but in many cases comparable to that of *P. patula*. In 21 cases, comparisons were recorded across a range of sites and with a variety of sources representing both the hybrid and the *P. patula*. In seven cases, the survival of the hybrid and *P. patula* was comparable; in three cases, the hybrid had better survival and in the remaining eleven cases, the survival was higher for *P. patula*.

Overall the survival of the *P. patula* × *P. tecunumanii* hybrid was generally poorer than *P. patula* during this period. These plantings were established in the late 1990s when the field survival related to *Fusarium circinatum* was still not having a major impact on the stocking of *P. patula*. It is interesting to note however, that many of the situations where the survival of the hybrid was poorer than *P. patula*, can be associated with plantings made with physiologically older hedge material. In addition, during this period, cuttings of pure *P. patula* generally had a reputation for poorer survival, when compared to seedlings, and this would have applied to the hybrid cuttings as well. This was thought to be related to issues of cutting quality, lack of experience in handling/extracting cuttings at planting and other management issues related to the raising of cuttings from hedges. Finally, in some cases, the poorer survival could be attributed to poor survival in one (or more) particular hybrid seedling families. Thus, in many cases it was possible to explain the poorer survival of the hybrid. The results from these trials therefore that, provided propagation protocols were followed, the hybrid could be established successfully on both the warm and cold temperate sites in South Africa.

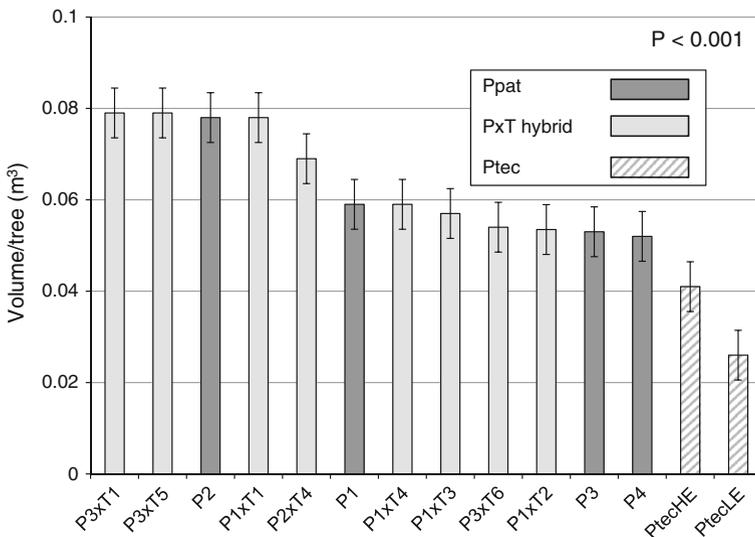


Fig. 1 Mean tree volume for a number of *P. patula* and *P. patula* × *P. tecunumanii* (Napite and Pachoc, high elevation provenances) hybrid families at 5 years on a medium elevation site. P and T numbers refer to specific families of *P. patula* and *P. tecunumanii*—open pollinated families of all *P. patula* were included

Tree growth

During this phase of testing growth comparisons were possible across a large range of sites (Table 4) and with hybrids where the *P. tecunumanii* pollen parent was sourced from a number of different provenances representing both high and LE material. In all cases, at least some *P. patula* × *P. tecunumanii* hybrid families were comparable or better than commercial *P. patula*. A fairly ‘typical’ example, selected because it represents a number of hybrid and *P. patula* families and was planted as seedlings in a replicated hybrid seedling progeny trial is presented in Fig. 1 (Kanzler 2006a).

The range of comparisons across these sites suggested that the relative performance of the *P. tecunumanii* hybrid source (low—(LE) or high—(HE) elevation) had an impact on the performance based on altitude. The trend was for the *P. patula* × *P. tecunumanii* (LE) hybrids to generally perform better on lower, warmer sites and vice versa for the *P. patula* × *P. tecunumanii* (HE) hybrids.

Stem form and forest ‘acceptability’

Pinus tecunumanii has a reputation for stem breakage problems (Dvorak et al. 1993) and it would be important to assess the extent of this occurrence within the hybrid. Stem form, including stem breakage, was assessed in a number of different ways. In a number of trials a stem form score was given for each tree, based on a scale of one to five, whilst in other tests, any stem defects such as forking, stem breakage and foxtailing was taken as an indication of the level of stem form defect. Overall, the stem form, when comparing the *P. patula* × *P. tecunumanii* hybrid to the parent species, was very variable and was dependant on a number of factors. These included: the source of *P. tecunumanii* used as a parent, the specific cross, the site and the management practice.

The source of *P. tecunumanii*, and perhaps more importantly, the exact nature of the cross was found to play a primary role. In one specific example a single source of *P. tecunumanii* was crossed to five different *P. patula* parents and both the highest and lowest number of stem defects was recorded amongst the *P. patula* × *P. tecunumanii* hybrid families. Clearly, by testing and selecting carefully it would be possible to find good crosses that were as good, or better, than the commercial *P. patula* control.

Pest and diseases

One of the major advantages of the hybrid was the increased tolerance to the pitch canker fungus (PCF), *F. circinatum*, over that of *P. patula*. In seedling screening trials, *P. tecunumanii* was found to be substantially more tolerant to *F. circinatum* than *P. patula*, with the LE *P. tecunumanii* more tolerant than the high elevation material (Hodge and Dvorak 2000).

A series of three tests in South Africa, utilising 3-year old field trials and with a range of Pine hybrids, including the *P. patula* × *P. tecunumanii* (Culmi) hybrid was subjected to branch inoculations with the PCF strain currently found in South African nurseries (Roux et al. 2007). The rankings of the various species and hybrids were consistent with expectations derived from the seedling screening trials with the *P. patula* × *P. tecunumanii* hybrid ranked higher than the pure *P. patula* entries (Hodge and Dvorak 2000).

In terms of other pest or diseases, little other information is known. Generally, the hybrid was found to be healthy and free of any obvious insect or pathogens.

Wood and pulping properties

In terms of wood properties, *P. tecunumanii*, grown in South Africa, generally has higher wood density than *P. patula*, *P. elliottii* and *P. taeda* and the LE provenances have slightly higher wood densities than the high elevation provenances (Dommissie 1994; Malan 1994; Wright et al. 1987). A study on 17-year old trees of *P. tecunumanii* in Mpumalanga showed that the percentage latewood was less and the earlywood density higher than *P. taeda* and *P. patula*, grown on the same site (Malan 1994). This resulted in wood that was markedly less variable in density in both the radial and axial stem directions. This finding has been confirmed in another study across three sites in Swaziland, where several provenances from both the high and LE sources were assessed (Kanzler 2006b).

Currently, little is known about the wood properties of the *P. patula* × *P. tecunumanii* hybrid. Most hybrid tests have not reached an age where reliable inferences around the wood properties can be made. A study comparing the wood properties of 9-year old *P. patula*, *P. tecunumanii* and *P. patula* × *P. tecunumanii* high elevation (Las Piedrecitas) hybrid across three sites in Swaziland showed that the hybrid had intermediate wood densities to that of the parent species (Kanzler 2012). Furthermore, although not statistically significant, the hybrid would also be expected to have more uniform wood than *P. patula* with lower latewood and higher earlywood densities (Fig. 2).

General conclusions from phase 1

Overall, the results of all the studies conducted in 1990's and early 2000's (Table 2) supported the idea that the *P. patula* × *P. tecunumanii* hybrid could be a viable commercial taxon for Southern Africa and a replacement on many sites for *P. patula*. The following broad conclusions from this phase were:

1. There was some evidence that the frost tolerance of the *P. patula* seed parent was inherited by the hybrid. This applied to both the high and LE sources of *P. tecunumanii*.

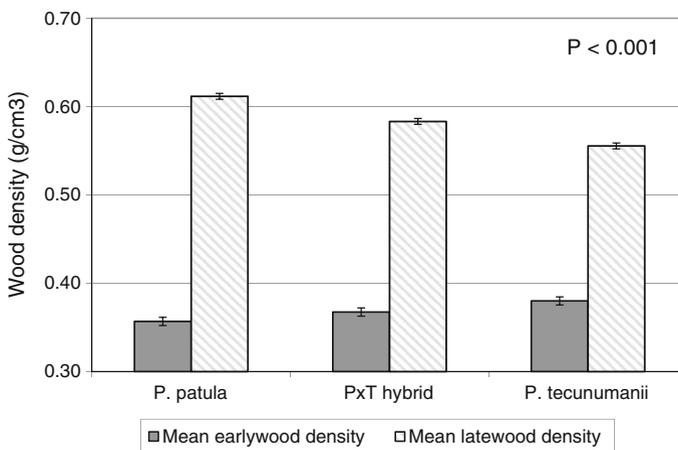


Fig. 2 Mean early and latewood densities for *P. patula*, *P. tecunumanii* and the hybrid (P × T hybrid) at 9 years of age, across three sites, varying in elevation from 1,060 to 1,600 m

2. The *P. patula* × *P. tecunumanii* hybrid cuttings could be propagated and deployed successfully as cuttings, although they did appear to demonstrate similar physiological ageing effects to that of pure *P. patula*.
3. Good field survival for the *P. patula* × *P. tecunumanii* hybrid was possible, provided current protocols developed for propagating *P. patula* cuttings were followed.
4. A number of different *P. patula* × *P. tecunumanii* hybrid crosses from a range of *P. tecunumanii* provenances, representing both high and LE sources have been grown successfully on a broad range of sites currently planted to softwoods within South Africa.
5. There was a large amount of variation in growth amongst the hybrid material tested. A significant source of this variation in productivity was at the family level. It seems likely that it would be important to test individual full-sib crosses comprehensively before any operational deployment.
6. There was some evidence that the broad adaptability of the *P. patula* × *P. tecunumanii* hybrid was dependent on the source of the *P. tecunumanii*. The high elevation sources tended to perform better on higher, cooler sites whilst the LE material performed relatively better on the warmer, LE sites.
7. The overall stem form of the *P. patula* × *P. tecunumanii* hybrid was intermediate between the two parent species but here again variation was found at the family level.

Fusarium circinatum as a threat to *P. patula*

Fusarium circinatum was first discovered in a South African nursery in 1991 (Viljoen et al. 1994). It soon spread to all pine growing nurseries in South Africa, causing large scale mortality of nursery seedlings, especially in *P. patula* (Mitchell et al. 2011). The disease is now managed in commercial nurseries with strict hygiene practices, and mortality of seedlings has been drastically reduced (Morris 2010; Mitchell et al. 2011, 2012a). During the last 10 years, however, the disease started to manifest in the field, impacting on post-planting survival (Crous 2005). The propagation of *P. patula* by means of cuttings has also become extremely problematic due to the fact that hedge plants are particularly susceptible to infection (Mitchell et al. 2011), so much so that vegetative propagation of high value *P. patula* has been rendered uneconomic (L. Williams pers. comm., Sappi nursery, 2009). Pitch Canker may also be a threat to mature trees in plantations, and in fact has been positively identified in 10 year old *Pinus radiata* grown in the Western Cape (Coutinho et al. 2007). The financial loss due to the increased establishment costs caused by *F. circinatum* is estimated to be around R11 million South African Rands annually (Mitchell et al. 2011). The disease has also caused a large decrease in grafting success, which has negatively impacted on tree improvement activities and clonal seed orchard establishment.

Artificial seedling inoculation trials of various pine species have demonstrated differential responses to *F. circinatum*, and indicated that *P. patula* has low to intermediate tolerance (Hodge and Dvorak 2000). *P. tecunumanii* on the other hand, displayed high levels of tolerance to *F. circinatum* (Hodge and Dvorak 2007). The intermediate tolerance of *P. patula* and high levels of resistance of *P. tecunumanii* has also been confirmed in a study where pine species such as *Pinus maximinoi*, *Pinus elliottii*, *Pinus pseudostrobus* and *P. tecunumanii* were evaluated, and *P. patula* was included as a control (Mitchell et al. 2012b). In-field inoculations of 2–3-year old trees of various *Pinus* species and hybrids also indicated that *P. patula* was susceptible to *F. circinatum*, with increased tolerance of the *P. patula* × *P. tecunumanii* hybrid (Roux et al. 2007). In addition, further nursery

screening studies have also shown increased tolerance of the *P. patula* × *P. tecunumanii* hybrid to *F. circinatum* (Mitchell et al. 2012c). This increase in tolerance of the *P. patula* × *P. tecunumanii* hybrid and the large scale economic losses associated with *P. patula*, provided the impetus for the rapid development and commercialization of the hybrid in South Africa.

Rapid development and deployment of the *P. patula* × *P. tecunumanii* hybrid

The primary focus of this second phase in the development of the hybrid (Table 2), has been on crossing *P. patula* with the LE *P. tecunumanii* sources because of the greater *Fusarium circinatum* tolerance of these populations over those from high elevation (Hodge and Dvorak 2000, 2007). This second phase was underpinned by research into factors controlling successful controlled pollination of *P. patula* (Nel 2002; Nel and van Staden 2003, 2005), and investment in pollen processing facilities. In addition, a large scale project was initiated to produce and test a range of alternative pine hybrids. This latter project is referred to as phase 3. Both of these phases commenced during the mid-2000s and continue to the present time (Table 2).

Discussion of the polymix mating design and clonal testing approach

It was decided to use a polymix mating design to generate the crosses for this next phase for a number of reasons:

1. It was necessary to produce a set of hybrid crosses using selected *P. tecunumanii* pollen in as short a time as possible. Controlled pollination (CP) is expensive and requires large resources. This is even more problematic using *P. patula* as a maternal parent because of the low cone survival figures generally reported by South African tree breeders (Nel and van Staden 2003). The polymix approach allowed Sappi to generate a large number of hybrid families (twenty-five), by making just five CP crosses, using a selected 5-parent *P. tecunumanii* pollen mix. In practice, these CP mating designs were completed in 1 year (2003), the seed was harvested in 2005 and sown in 2006.
2. In order to 'reconstitute' the pedigree of the pollen parent it was necessary to maintain clonal identity. Each individual seed was sown and then maintained as a seedling hedge. The cost effective development of pine DNA markers during this period has made this approach possible.
3. The testing program thus involved clonal testing as a key part of the strategy (Nel and Kanzler 2013). This approach meant that the potential gains in utilising clones could be measured accurately which provided a good foundation for estimating the benefits of investing in clones through somatic embryogenesis.

Crosses made and fingerprinting

Polymix crosses were completed in 2003 using five *P. patula* parents from a 2nd generation clonal seed orchard, and two five-parent *P. tecunumanii* low elevation polymix pollen lots. A total of six polymix families were produced with a potential of producing 30 different specific full-sib hybrid crosses, provided that all pollen lots in the polymix were successful during the pollinations. Seed was sown in 2006 with objective of establishing

one hundred seedling hedges per polymix cross (under a scenario of an equal representation of the five specific male parents it was hoped that this would yield around twenty individual hedges per hybrid family), giving a total of 600 hedges. In practice, after DNA finger-printing each hedge, the results revealed 26 specific crosses, with some unexpected crosses, as result of incorrect labelling of ramets in the breeding bank (Nel and Kanzler 2013).

Testing strategy and discussion

The testing of all the rooted cuttings produced from the 600 seedling hedges took place over 5 years (2007–2011) and involved a number of different tests—field plantings consisted of a combination of unreplicated block plantings and replicated clonal tests as well as nursery *F. circinatum* clonal screening trials.

Clonal field tests

Rooted cuttings were produced from seedling hedges of the six *P. patula* × *P. tecunumanii* LE polymix families. Individual seedling identity was retained for all rooted cuttings for every hedge, creating clonally identical rooted cuttings from every individual seedling. Fingerprinting results revealed the actual specific cross for each seedling-hedge, and each specific hybrid family was represented by differing numbers of clones.

A number of large clonal field tests (six), with these cuttings, was established across a range of sites (Nel and Kanzler 2013). These trials consisted of 100–450 clones tested in four or six tree line plots with three to four replications. The objective of these trials was to accurately estimate the clonal variation and by retrospectively determining the full pedigree, obtain some estimates of general hybridising ability (GHA) and specific hybridising ability (SHA).

Block plantings

An unreplicated block planting testing strategy was implemented to complement the replicated clonal field tests. These unreplicated block plantings, consisted of three 7 × 7 tree plots, representing three hybrid polymix families, where each block was constituted as seven clones in seven tree line plots. In addition, each planting also included a 7 × 7 tree square block of a 2nd generation *P. patula* bulk seed orchard seedlot that was currently being deployed commercially. All four treatments were managed equally, no replanting was done to recover seedling mortality occurring in the first few months following site establishment.

It was clearly recognized that any inferences for the individual clone and hybrid polymix family per site was very limited. However, the advantages of this approach were as follows:

1. The material from each seedling hedge was being produced continuously and erratically—opportunistic block plantings meant that the vegetative material being produced could be used efficiently and effectively;
2. A large number of sites could be established, rapidly and cost effectively—twenty sites over 3 years;
3. *Pinus patula* and the hybrid could be compared across a large number of sites, years and seasons.

4. The low cost and ease of establishment meant that these plantings could be placed on sites associated with a high degree of risk—for example, drought and frost;
5. It gave the opportunity to observe clonal differences, although perhaps not accurately quantify them.

Nursery *Fusarium circinatum* screening trials

Ten-month old rooted cuttings, using the same material as outlined above, with clonal identity retained were utilized in a number of nursery *F. circinatum* inoculation trials, carried out at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Inoculation protocols originally developed by Oak et al. (1987) were used for the inoculation studies, with some adaptations described in Porter et al. (2014) and Nel et al. (2014). Lesion lengths caused by the *F. circinatum* inoculations were assessed 12 weeks after inoculation and the data was analysed using methods described by Nel et al. (2014).

Propagation of *P. patula* × *P. tecunumanii* for the phase 3 study

Various pure and hybrid taxa, and families within each, were sown into Unigro 98™ trays in January 2008 at the Sappi Shaw Research Centre located at Tweedie, South Africa. The seedlings were grown out under cover, on tables until approximately 8 months old. Seedlings were then planted into 4 l polythene bags of composted pine bark. Seven different taxa were tested (see Fig. 3), including two sources of *P. patula* and six pine hybrid combinations. Each taxa was represented by between three and six families and around 200–800 seedling hedges. Rooted cuttings from each of the hedges were produced between October 2008 and March 2012. Twenty-two settings of this material were harvested and rooted in greenhouse conditions. Two settings were excluded from the data analysis because they experienced system failures during rooting. Clonal identity of the mother hedge for each of the cuttings was maintained. Production and rooting data was recorded at the clonal level.

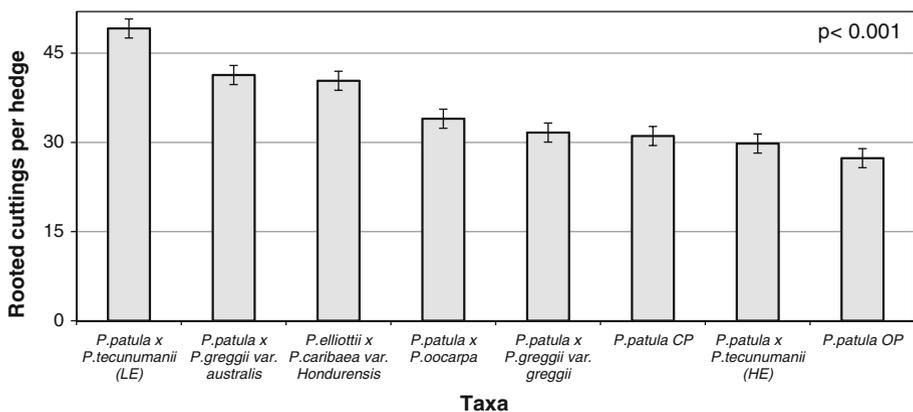


Fig. 3 Number of rooted cuttings established per hedge from the pure and hybrid taxa over a 42 month period (twenty settings), from 2008 to 2012 [reproduced with permission from Ford et al. (2013)]

Results and discussion from phase 3

Hedge productivity in nursery

Rooting of the various *P. patula* hybrids was almost as good as the *P. elliotii* × *P. caribaea* hybrid and were comparable or better than the rooting in the pure *P. patula*. The *P. patula* × *P. greggii* var. *australis* and *P. patula* × *P. tecunumanii* (LE) hybrids rooted significantly better than parental *P. patula* but there was no difference in the rooting of the *P. patula* produced by controlled and open pollination. The number of rooted cuttings produced per hedge, was highest in the *P. patula* × *P. tecunumanii* (LE) hybrids followed by the *P. patula* × *P. greggii* var. *australis* and *P. elliotii* × *P. caribaea* hybrids (Fig. 3). Open pollinated *P. patula* produced less rooted cuttings than the controlled pollination source.

Fusarium circinatum tolerance

Dying hedges in the nursery, during the period 2008–2012, were collected and sent to the Forestry and Agricultural Biotechnology Institute's TPCP diagnostic clinic at the University of Pretoria for molecular confirmation of infection with *F. circinatum*. Figure 4 shows that *F. circinatum* related mortality in open pollinated *P. patula* was significantly worse ($P < 0.001$) than that observed in the control pollinated *P. patula* (selected for growth and tolerance) and in the *P. patula* × *P. tecunumanii* (LE) hybrid, which were 32, 17 and 9 % respectively (LSD = 9.98 %). The *P. patula* hybrids with *P. greggii* and *P. tecunumanii* (HE) also showed significantly higher mortality through *F. circinatum* than the *P. patula* × *P. tecunumanii* (LE) hybrid.

Results from a *F. circinatum* nursery inoculation experiment are shown in Fig. 5. Tolerance to *F. circinatum* was assessed by measuring the lesion development from the

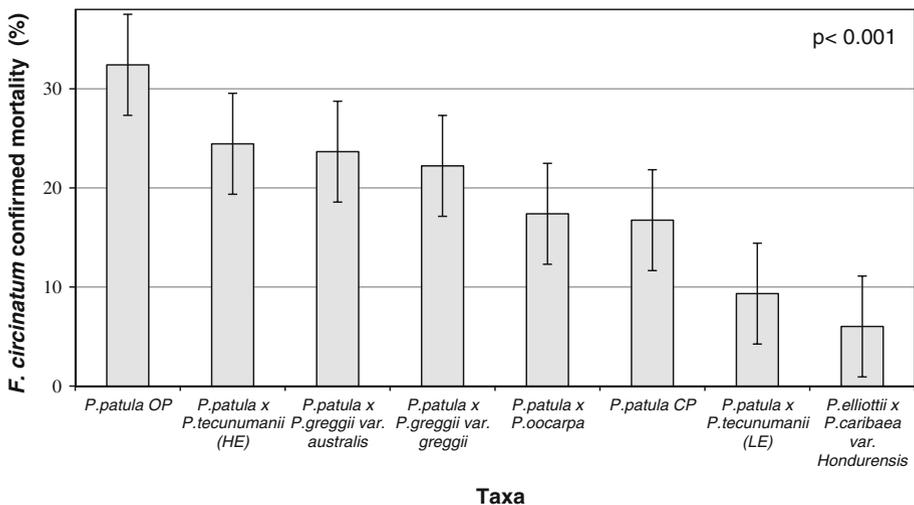


Fig. 4 Species hedge mortality in the production trial which could be positively linked to infection with *F. circinatum* (reproduced with permission from Ford et al. (2013))

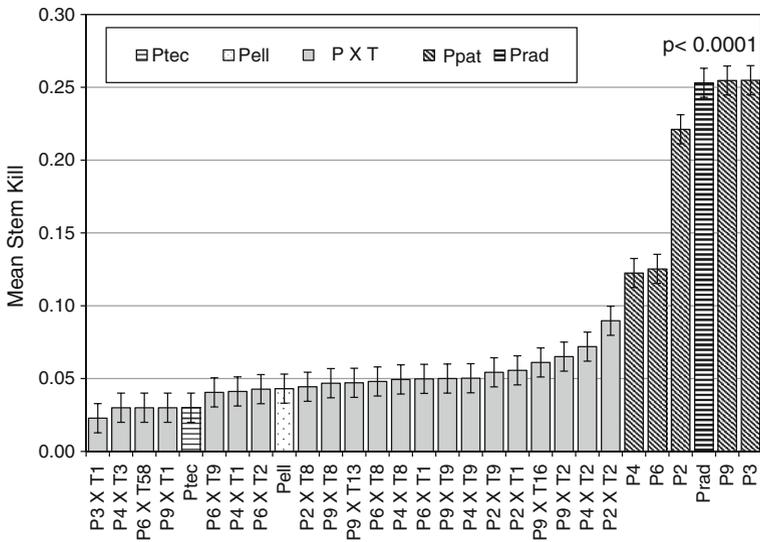


Fig. 5 Mean stem kill (lesion length over plant height) results from two *F. circinatum* nursery inoculation experiments of a number of DNA finger-printed specific *P. patula* × *P. tecunumanii* low elevation hybrid families, *P. patula* hybrid parents, and other species controls such as *P. tecunumanii* (Ptec), *P. elliotii* (Pell) and *P. radiata* (Prad). Lower stem-kill values indicate higher tolerance and highly significant differences between treatments were noted. P and T numbers refer to specific families of *P. patula* and *P. tecunumanii*

top, along the stem of inoculated plants, 8 weeks after inoculation, expressed as a proportion of the total length of the plant stem (stem-kill). Results indicate that the control species *P. tecunumanii* LE and *P. elliotii* had stem-kill proportion values lower than 4 %, indicating high levels of tolerance to *F. circinatum*. The various *P. patula* open-pollinated hybrid parent selections, together with the *P. radiata* species control had the highest stem-kill values, ranging between 12 and 26 %. The various *P. patula* × *P. tecunumanii* LE hybrid specific families ranged between the *P. tecunumanii* LE and *P. patula*, with stem-kill values of between 2 and 9 %. Most hybrid families displayed stem-kill values which were closer to the *P. tecunumanii* than the *P. patula* parent species.

Establishment and survival

The survival at 9 months of both the commercial *P. patula* and the *P. patula* × *P. tecunumanii* LE hybrid was assessed across thirteen sites within the block plantings. At each site, all treatments received the same management. Survival was generally better for the hybrid than the pure species, but it was apparent that on certain sites, especially those where frost damage was recorded, the hybrid performed more poorly than the pure species. Averaged across all sites, but excluding the sites where it could be verified that mortality in the hybrid was associated with frost, the hybrid had between 10 and 15 % higher survival than the *P. patula*.

Growth and stem form

Volume growth at 4 years was assessed across six sites in the oldest block plantings planted during 2007. These six sites included a large range in elevation, ranging from

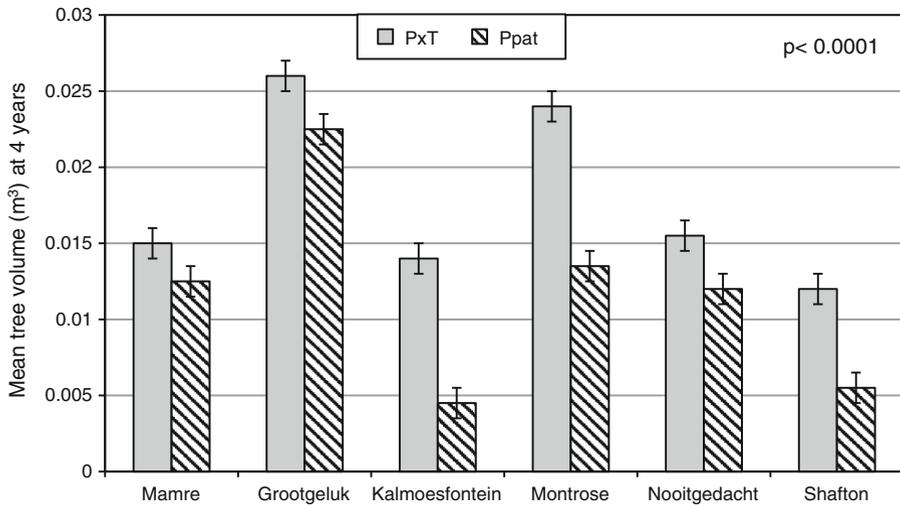


Fig. 6 Four-year volume growth of *P. patula* and *P. patula* × *P. tecunumanii* (low elevation) hybrid over six sites planted during 2007

Mamre (1,770 m), Grootgeluk (1,481 m), Kalmoesfontein (1,358 m), Montrose (1,322 m), Nooitgedacht (1,251 m) to Shafton (1,180 m). Heights and diameter at breast height were assessed for all trees and mean tree volume for these six sites are presented in Fig. 6. The *P. patula* × *P. tecunumanii* LE hybrid was more productive on all six sites and the average growth improvement over *P. patula* was 46 % at 4 years.

Stem form, and in particular stem breakage problems were assessed in both the block plantings and clonal trials. Generally the stem form was excellent and little evidence of stem breakage, or other problems, were encountered.

Cold/frost tolerance

The commercial *P. patula* plantations are restricted to the summer rainfall areas of Southern Africa, at latitudes between 23 and 30°S, and mean annual temperatures less than 17 °C with the species being characterised as having relatively good frost tolerance (Morris and Pallett 2000). In its native habitat the species occurs in a montane belt from 16 to 24° latitude with temperatures on many sites dropping to well below zero from 5 to 101 days per year (Dvorak et al. 2000a). The LE *P. tecunumanii*, on the other hand, occurs at elevations below 1,500 m and latitudes between 13 and 17°. These populations are unlikely to experience much frost at all (Dvorak et al. 2000b). Minimum temperatures for these two species have been estimated as −9 °C for *P. patula* and 0 °C for LE *P. tecunumanii* (Dvorak, pers. comm.). Given the more tropical nature of the latter parent, some frost susceptibility in the hybrid would be expected.

A description of frost and cold problems in South Africa

The plantation sites currently planted to *P. patula* are situated at higher elevations and are often associated with mountainous terrain. The cold events in South Africa are linked with calm night air, clear sky and low dew-point temperature and involve radiation frost. Cold

air drainage results in the most severe frosts occurring in areas where the cold air accumulates (commonly referred to as “frost pockets”). Between 20 and 40 frost events (i.e. minimum temperature below 0 °C) occur per annum along the southern and south-eastern escarpment areas (Schulze and Maharaj 2007). Rapid and sudden drops in temperatures can occur from May through to September and diurnal fluctuations of more than 20–30 °C are not uncommon. Frosts either early in the autumn (May/June) or spring (September) are often associated with freeze damage.

*A comparison between *P. patula* and the hybrid from field trials*

Tree mortality as a result of frost has been identified over a number of sites where the hybrid has been planted alongside *P. patula*. At two of the sites, Helvetia and Nooitgedacht, the survival of the hybrid after a documented frost event was substantially poorer than the *P. patula*. Electronic data loggers were placed on both sites for a period of 4 months during winter and temperatures were recorded hourly. Minimum night time temperatures on the Helvetia site, dropped to around –8 °C on two consecutive days and substantial mortality amongst the hybrid genotypes was recorded. Nevertheless, clear differences between clones within the hybrid polymix families were apparent, clonal lines in adjacent plots differed in survival from 0 to 100 %, demonstrating that genetic differences for cold tolerance within the *P. patula* × *P. tecunumanii* LE hybrid were present.

The higher frost susceptibility of the hybrid presents a key challenge in the commercialisation of the hybrid as a replacement for *P. patula*. Two approaches are possible in managing this risk; utilise the genetic variation present and attempt to breed for frost tolerance or avoid areas with a high degree of frost risk by identifying and mapping these sites carefully. Both of these strategies have been utilised.

Evidence of genetic variation in frost tolerance

In order to explore the genetic component for frost tolerance, seed from a number of families of both *P. patula* and *P. tecunumanii* parents, as well as specific hybrid families were screened for frost tolerance using the electrolyte leakage method as described in Hodge et al. (2012). The results of this study showed that the frost tolerance of the hybrid families was intermediate between the two parent species and that the *P. patula* parents contributed most of the variation in frost tolerance among the hybrids (Cerdeira 2012).

Mapping of frost risk

Night time land surface temperatures derived from MODIS (MOD11A1 product) were used to determine the occurrence and severity of frost for all afforested areas in South Africa at a 1 km × 1 km pixel resolution. Frost occurrence was expressed as the number of days that the surface temperature for a particular pixel was below 0 °C (Pouteau et al. 2011) and the frost severity was calculated using the absolute minimum temperature of a pixel (Lindkvist and Chen 1999). Frost maps derived from satellite data are useful for identify regional patterns of risk (Pouteau et al. 2011) but are too coarse for the investigation of frost risk at a local compartment scale. For this reason additional topographical variables derived from a 20 m × 20 m digital elevation model were incorporated to account for the possibility of localized cold air accumulation (Lindkvist et al. 2000). Preliminary results are meaningful and further research is being implemented to verify and

operationalize the compartment scale model. This accurate mapping of forest land has allowed Sappi to deploy the hybrid more precisely and reduce the risk of frost damage.

General conclusions

The extensive research and testing documented in this paper, comparing *P. patula* with the *P. patula* × *P. tecunumanii* hybrid has shown that the hybrid has;

- Better tolerance to *F. circinatum* and improved post-planting survival
- Superior volume growth, with a 46 % improvement at 48 months
- An effective and economic propagation method
- Acceptable wood and pulping properties with similar wood density to *P. patula*
- Large clonal differences that may be exploited via somatic embryogenesis
- Greater tolerance to warmer sites
- Greater susceptibility to frost damage that impacts on survival on some sites, although genetic differences in frost tolerance are apparent and can be exploited

In summary the *P. patula* × *P. tecunumanii* (LE) hybrid has performed well across the entire range of sites within the temperate zone in Southern Africa and is a viable and economically beneficial replacement for *P. patula* on many sites.

A discussion of future considerations

The operational deployment of the *P. patula* × *P. tecunumanii* hybrid has been approached (and structured) by defining four phases (Table 2). Phase 4 involves the production and testing of a large number of additional hybrid families through a large factorial mating design. This phase is currently underway and will result in a broader, more sustainable deployment of this hybrid into the future and across the large range of temperate sites. In addition, the actual environmental niches of the *P. patula* × *P. tecunumanii* (LE), *P. patula* × *P. tecunumanii* (High elevation) and *P. patula* taxa needs to be more clearly defined by extensive and careful site × Taxa (species and hybrid) testing. In the medium term, *P. patula* may not be entirely replaced by the hybrid because there are some sites that experience severe frost events.

Breeders should also take cognisance of the many other challenges that southern African plantations will face in the next few years. These include global climate change with the resultant changes in the sub-region (Davis 2011), increasing incidence of pests and diseases (Wingfield et al. 2010) as well as changing markets and expansion of plantations into new areas in Africa and elsewhere. Thus they will need to constantly be looking for new opportunities to exploit and effectively utilise the variation available to them, including the possibility of other hybrid combinations. One future consideration which has not been addressed, that should be considered by southern African softwood breeders is briefly explored below.

During the last 30 years, many organisations in the sub-region have been acquiring and testing substantial breeding material from a range of closed cone pines with the help of Camcore and others (Dvorak 2000). These species include *P. patula*, *P. tecunumanii*, *P. greggii*, *P. pringlei*, *P. jaliscana* and *P. oocarpa*. In all of these species the material has been acquired in a structured way that includes both provenance and family identity. The result is that variation at the species, provenance and family level has been quantified for

many economically important traits such as growth (Hodge and Dvorak 2012), frost tolerance (Mitchell et al. 2013; Hodge et al. 2012), wood properties (Camcore Annual Report 2008a, b; Stanger 2003) and *F. circinatum* tolerance (Hodge and Dvorak 2000, 2007). It is suggested that this variation on a species (and provenance) level should be utilised in future hybrid mating designs. In this way it may be possible to understand and quantify hybrid genetic parameters on a species and provenance (within species) level. In addition, it has recently been suggested (de Resende and de Assis 2008) that making further ‘hybrid advanced generation’ crosses amongst selected hybrid trees, or backcrosses to specific populations to create ‘synthetic populations’ might be advantageous. The wide genetic diversity in pines makes this an intriguing possibility for southern African tree breeders.

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