# Susceptibility of provenances and families of *Pinus maximinoi* and *Pinus tecunumanii* to frost in South Africa

**R. G. Mitchell · M. J. Wingfield · G. R. Hodge · W. S. Dvorak · T. A. Coutinho**

**Abstract** The future of South Africa's most important pine species, *Pinus patula*, is threatened by the pitch canker fungus, *Fusarium circinatum*. *Pinus maximinoi* and *P. tecunumanii* represent two subtropical species that provide an alternative to planting *P. patula* on the warmer sites of South Africa. Extending the planting range of *P. tecunumanii* and *P. maximinoi* to include higher and colder altitude sites will reduce the area planted to *P. patula* and the risk of *F. circinatum*. During 2007 progeny trials of *P. tecunumanii* and *P. maximinoi* were planted on a sub-tropical and sub-temperate site. Shortly after the establishment of these trials, unusually cold weather conditions were experienced across South Africa ($-3°C$ at the sub-temperate site) resulting in severe mortality. This provided the opportunity to assess the variation in survival as a measure of frost tolerance within these two species to determine whether it could be improved upon through selection. Results indicated that the variation in survival was under genetic control in *P. tecunumanii* ($h_{(0,1)}^2 = 0.16$, $h_L^2 = 0.27$) and *P. maximinoi* ($h_{(0,1)}^2 = 0.11$, $h_L^2 = 0.23$) at the sub-temperate site. Correlations in provenance ranking for survival across sites were high for both species. Moderate correlations in family survival for *P. tecunumanii* ($r = 0.52$) were found at the two sites. Improvements in cold tolerance can thus be made in both species extending their planting range to include greater areas planted to *P. patula* thereby limiting the risk of *F. circinatum*.

**Keywords** Camcore · Genetic diversity · Frost susceptibility · South Africa · *Pinus maximinoi* · *Pinus tecunumanii*

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## Introduction

There is considerable interest in commercializing alternative pine species not commonly planted in South Africa. This emerges from a desire to improve several weaker characteristics of currently deployed species, including the susceptibility of *Pinus patula* to the pitch canker fungus, *Fusarium circinatum*. *Pinus tecunumanii* and *Pinus maximinoi* are two alternative species that have shown good growth (Kietza 1988; Dvorak et al. 2000a, b; Gapare et al. 2001), wood properties (Malan 1994, 2006), and tolerance to *F. circinatum* (Hodge and Dvorak 2000, 2007; Mitchell et al. 2011). *P. tecunumanii* may be a particularly valuable species because it hybridises easily with South Africa's most important pine, *P. patula*, resulting in improved tolerance of the hybrid to *F. circinatum* (Roux et al. 2007).

Pinus tecunumanii and *P. maximinoi* are susceptible to frost that limits their planting to warmer sites. Populations of *P. tecunumanii* can be divided into those that occur below 1,500 m altitude (low elevation subgroup) and those that occur above 1,500 m (high elevation subgroup) in their natural origin in Central America and Mexico (Dvorak 1986; Dvorak et al. 2000a). High and low elevation subgroups of the species also are distinguishable genetically through molecular marker assessment (Dvorak et al. 2009). From a climatic standpoint, low elevation provenances are more tropical in nature and particularly sensitive to frost while high elevation provenances from southern Mexico and Guatemala can tolerate light frost (Dvorak et al. 2000a). Similarly, *P. maximinoi* grows best on tropical or sub-tropical sites free from frost (Dvorak et al. 2000b). Improving the frost tolerance of these two species would increase their planting range to include warm and sub-temperate sites currently planted to *P. patula*.

During 2007, progeny trials of *P. tecunumanii* and *P. maximinoi* were established just before a winter period. The survival of these trials was severely affected by a frost event resulting in their termination. In this study the survival data were examined and tolerance of these two species to frost was determined.

## Methods

A group of 113 *P. tecunumanii* and 43 *P. maximinoi* trees (hereafter called "selections") were identified in 1st generation provenance/progeny trials planted by Komatiland Forests. Selection criteria were provenance and family growth, and individual tree growth and form. The trials were then heavily thinned, effectively converting the progeny tests into seedling orchards. Seed was collected from the selections, and was sown at the Komatiland Forest's nursery near Sabie in September 2006. Sufficient seed was sown to plant two second-generation trials of each species on two separate predetermined sites (Spitskop and Wilgeboom). The Spitskop site could be described as sub-temperate while the Wilgeboom site is sub-tropical. The 1st generation selections represented a number of the original provenances from different countries in Central America and southern Mexico (Table 1), which had been planted in blocks. The selections were, therefore, likely to have been pollinated by surrounding trees of the same provenance, but may also have been pollinated by trees further away and not necessarily of the same provenance.

Seedlings were raised in 128 Unigro plastic molded trays with an individual cavity size of 60 cc. Well-composted pine bark was used as the growing medium and 2:3:2 (22) N:P:K granular fertilizer was applied as required.

The trials were planted in mid-March 2007 when seedlings were 6-months-old. At each site, one *P. maximinoi* and one *P. tecunumanii* trial was planted (Table 2). Based on the

**Table 1** Number of families representing provenances

| Species | Ecotype | Provenance | State | Country | Wilgeboom | Spitskop |
|---|---|---|---|---|---|---|
| *Pinus maximinoi* | NA | Coban | Alta Verapaz | Guatemala | 6 | 5 |
| | | San Jeronimo | Baja Verapaz | Guatemala | 10 | 9 |
| | | San Juan Sacatep. | Guatemala | Guatemala | 6 | 5 |
| | | San Lorenzo | Zacapa | Guatemala | 1 | 1 |
| | | Copan | Copan | Honduras | 3 | 2 |
| | | Tatumbla | Fco. Morazán | Honduras | 3 | 3 |
| | | Marcala | La Paz | Honduras | 3 | 2 |
| | | El Portillo | Ocotepeque | Honduras | 3 | 3 |
| | | Altamirano | Chiapas | Mexico | 1 | 1 |
| | | La Canada | Chiapas | Mexico | 2 | 2 |
| | | Monte Cristo | Chiapas | Mexico | 1 | 1 |
| | | San Jerónimo | Chiapas | Mexico | 4 | 4 |
| Total | | | | | 43 | 38 |
| *Pinus tecunumanii* | High | San Jerónimo | Baja Verapaz | Guatemala | 7 | 4 |
| | High | KM 47 | Guatemala | Guatemala | 1 | 1 |
| | High | La Soledad | Jalapa | Guatemala | 1 | 1 |
| | High | San Lorenzo | Zacapa | Guatemala | 3 | 2 |
| | High | Las Trancas | La Paz | Honduras | 3 | 2 |
| | High | Chempil | Chiapas | Mexico | 13 | 9 |
| | High | Jitotol | Chiapas | Mexico | 3 | 3 |
| | High | Las Piedrecitas | Chiapas | Mexico | 2 | 1 |
| | High | Montebello | Chiapas | Mexico | 8 | 6 |
| | High | Napite | Chiapas | Mexico | 2 | 1 |
| | High | San José | Chiapas | Mexico | 2 | 2 |
| | Low | Jocon | Yoro | Honduras | 4 | 2 |
| | Low | San Esteban | Olancho | Honduras | 13 | 9 |
| | Low | San Francisco | Olancho | Honduras | 18 | 15 |
| | Low | Villa Santa | El Paraiso | Honduras | 33 | 26 |
| Total *P. tecunumanii* | | | | | 113 | 84 |

*NA* Not applicable

availability of seedlings, the Wilgeboom trials were comprised of the full set of families whilst the Spitskop trials contained fewer families. In the case of both species, the families in the Spitskop trials were common in the Wilgeboom trials. In each case, the trial design was a randomized complete block with 6 replications and 6 tree family row plots. Each family, therefore, was represented by 36 trees in each trial. In all the trials *Pinus elliottii* and *P. patula* seedlings from a commercial seed orchard were used as controls.

The trials were assessed for survival after 60 days and blanked to 100% stocking. Approximately 5 days after the blanking operation, extremely cold conditions were experienced across the country. The nearest weather station was at Graskop, a town approximately halfway between the two sites, and of similar altitude (1,450 m) and climate to the sub-temperate Spitskop site. Weather data for the month of May showed that
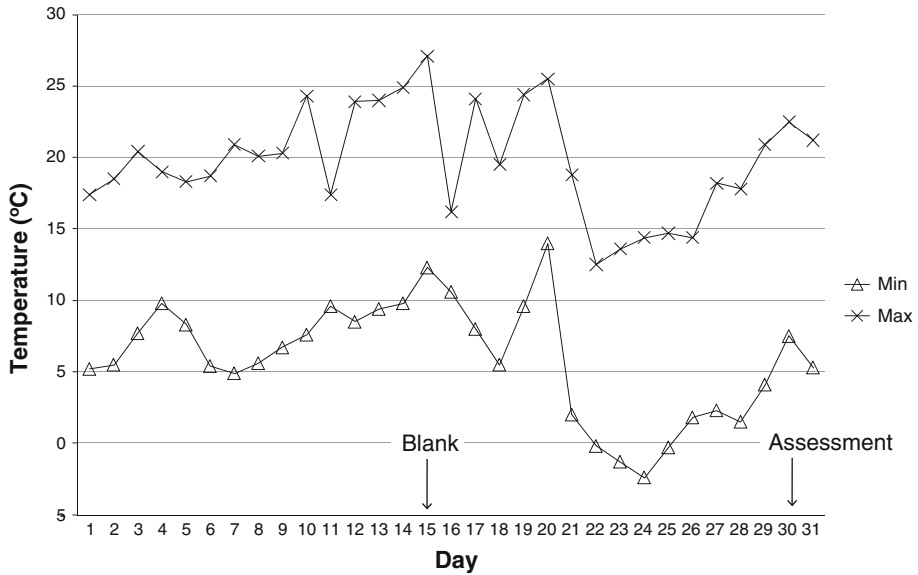
**Fig. 1** Minimum and maximum temperatures recorded, at a nearby weather station representative of the cool-temperate Spitskop site, for the month of May 2007

temperatures dropped to below freezing (0 to −3°C) for four consecutive days, and below 5°C for 9 consecutive days, between 21–29/05/07 at Graskop (Fig. 1). Soon after the frost event (75 days from the original planting), the trials were reassessed for survival. Severe mortality was recorded at the sub-temperate Spitskop site while less damage was recorded at the sub-tropical Wilgeboom site (Table 2).

## Statistical analyses

The statistical package SAS version 9.1.3 (SAS Institute 2004) was used to analyse the data in which case individual tree observations, recorded as dead (0) or alive (1), were used

**Table 2** Site details where the *Pinus maximinoi* and *Pinus tecunumanii* trials were planted in South Africa

| Trial site | Wilgeboom | Spitskop |
|---|---|---|
| Location | 30° 56′ 19″E; 24 57′ 5″S | 30° 50′ 23″E; 25 9′ 37″S |
| Description | Sub-tropical | Sub-temperate |
| Altitude | 983 m | 1,480 m |
| Mean min temperature in coldest month | 5°C | 4°C |
| Mean annual temperature | 18.5°C | 15°C |
| Mean max temperature in warmest month | 27°C | 24°C |
| Mean annual precipitation | 1,100 mm | 1,266 mm |
| Plant date (day 0) | 4–15/03/2007 | 12–13/03/2007 |
| Blank date (day 60) | 14/05/2007 | 15/05/2007 |
| Assessment date (day 75) | 30/05/2007 | 31/05/2007 |

as the unit of analysis. Several analyses of variance were conducted using SAS Proc GLM on each of the four individual tests (*P. tecunumanii* and *P. maximinoi* on the Spitskop and Wilgeboom sites). In all analyses, the binomial survival data was used as the units of observation.

Although inter-mating among trees from different provenances would have occurred in the thinned 1st generation progeny tests, the original provenance of a family could very likely have an impact on frost tolerance of the second-generation families. To investigate this, an analysis of variance was done to compare the control species, *P. patula* and *P. elliottii,* to the country and provenance of origin for *P. tecunumanii* and *P. maximinoi.* The linear model contained *rep* and *country* treated as fixed effects, and *provenance, family* (*provenance*), and *rep * family* (*provenance*) treated as random effects. SAS Proc GLM was used to conduct the analysis of variance, and least squares means (LS means) were calculated along with the p-values testing for statistically significant difference for each pair of means. A second GLM analysis was done using only the species data sets on each test site (that is, removing the control species), in order to test for differences among provenance and *family* (*provenance*). As above, in this model, *rep* was treated as fixed effects, while *provenance, family* (*provenance*), and *rep * family* (*provenance*) were treated as random effects, and LS means calculated as described above. A Pearson correlation was calculated between provenance and family means on the two sites for each species separately.

To examine the potential to breed for frost tolerance in the two species, genetic parameters for the populations were calculated with SAS Proc MIXED, using a linear model with *rep* treated as a fixed effect, and *family* and *rep * family* were treated as random effects. As the families were represented by open-pollinated seed collected in seedling seed orchards, converted from progeny tests, a coefficient of 3 provides a better estimate of additive genetic variance (Dieters et al. 1995). Heritability was calculated on the observed (binomial) scale, as $h^2_{(0,1)} = 3 \; \sigma^2_f/(\sigma^2_f + \sigma^2_{plot} + \sigma^2_{error})$ where $\sigma^2_f =$ estimated family variance, $\sigma^2_{plot} =$ estimated rep * *family* (*provenance*) variance, and $\sigma^2_{error} =$ estimated residual variance. The heritability estimate on the binomial scale was then converted to an estimate on the underlying liability scale ($h^2_L$) following the methodology of Chambers et al. (1996). Standard errors of the heritability were estimated using the approximation formula (Dickerson 1969), with the standard error of the family variance estimate calculated from the ASYCOV option in Proc MIXED.

## Results

Due to the more extreme temperatures, fewer plants survived at Spitskop than at Wilgeboom. The mean survival for *P. tecunumanii* (LE) was 27 and 46% for *P. tecunumanii* (HE) at Spitskop compared to 85.1 and 93.6%, respectively, at the Wilgeboom site. In the *P. tecunumanii* trial at Spitskop, *P. patula* survived better (83%) than *P. elliottii* (69%) compared to 100% survival for both species at the Wilgeboom site. The survival of *P. maximinoi* at the Spitskop site was 19% compared to 87% at Wilgeboom. In the *P. maximinoi* trials *P. patula* survival (89%) was poorer than *P. elliottii* (97%) at Spitskop and similar to *P. elliottii* at Wilgeboom (97 vs. 94%).

In the *P. tecunumanii* trials, the means of the HE families, which originated from southern Mexico and Guatemala, were significantly better than the mean of the LE families from Honduras at both sites (Table 3). The single high elevation variety of *P. tecunumanii* representing Honduras (Las Trancas) was significantly poorer than the mean of the high

| Species | Country | Spitskop (%) | Wilgeboom (%) |
|---------|---------|--------------|---------------|
| *Pinus tecunumanii* | *P. patula* | 83.3[A] | 100[A] |
| | *P. elliottii* | 69.4[A] | 100[A] |
| | Mexico (HE) | 52.2[B] | 92.0[A] |
| | Guatemala (HE) | 48.3[B] | 95.4[A] |
| | Honduras (HE) | 34.7[C] | 88.9[AB] |
| | Honduras (LE) | 26.6[C] | 85.3[B] |
| *Pinus maximinoi* | *P. elliottii* | 97.2[B] | 94.4[A] |
| | *P. patula* | 88.9[B] | 97.2[A] |
| | Honduras | 21.4[A] | 89.8[A] |
| | Mexico | 18.8[A] | 86.1[A] |
| | Guatemala | 16.3[A] | 85.5[A] |

**Table 3** The least square mean survival by country ranked from best to worst for *P. tecunumanii* and *P. maximinoi*

Treatments with different letters, for each species separately, are significantly different ($p < 0.05$)

elevation families from Guatemala and Mexico at the Spitskop site. In the *P. maximinoi* trials, families that originated from Honduras ranked better than those from Mexico that ranked better than those from Guatemala at both sites (Table 3). However, there were no significant differences between the means for each country at either site.

An analysis of provenance differences, which were represented by at least 2 families at a single site, indicated that the *P. tecunumanii* high elevation provenances (San Lorenso, Chempil, Montebello and San Jerónimo) survived significantly better than the *P. tecunumanii* low elevation provenances (San Esteban, San Francisco, Jocon and Villa Santa) at both the Spitskop and Wilgeboom sites (Table 4). Within the *P. tecunumanii* high elevation subgroup, the San Lorenso and Montebello provenances survived significantly better than the San José and Las Trancas provenances planted at the Spitskop site and the San José and Jitotal provenances at the Wilgeboom site (Table 4). Within the low elevation subgroup, the Villa Santa provenance survived significantly more poorly than the other LE provenances at the Spitskop site (Table 4). When comparing the ranking of provenances represented by at least 2 families at each site, there was a high correlation between the survival means on the two sites (Table 4).

There was no significant difference for all provenances of *P. maximinoi* at either site (Table 4), despite the fact that the LS means for survival ranged from 13.3 to 27.8 % at Spitskop, and from 83.8 to 93.5 % at Wilgeboom. Despite there being no significant differences between *P. maximinoi* provenances at either site, there was a high correlation between the survival means on the two sites (Table 4).

There was large family variation in survival at the Spitskop site for *P. tecunumanii* of both the low (3–61 %) and high (11–78 %) elevation subgroups. At the Spitskop site, a number of high elevation families showed little frost tolerance and some low elevation families showed frost tolerance similar to the mean of the high elevation variety (Fig. 2). The range of family survival in *P. maximinoi* at the Spitskop site was smaller, with the most tolerant family measuring 38 % survival (Fig. 3). There was little variation at the Wilgeboom site for both species and it is likely that only the most susceptible families showed some mortality. Narrow-sense heritability estimates for the trials at Spitskop were good for *P. tecunumanii* ($h^2_{(0,1)} = 0.16$, $h^2_L = 0.27$) and weaker for *P. maximinoi* ($h^2_{(0,1)} = 0.11$, $h^2_L = 0.23$) (Table 5). Narrow-sense heritability at the Wilgeboom site was poor for *P. tecunumanii* ($h^2_{(0,1)} = 0.05$, $h^2_L = 0.12$) and was nil for *P. maximinoi* (Table 5).

**Table 4** The least square mean survival by provenance (represented by at least 2 families) of *P. tecunumanii* and *P. maximinoi* ranked from best to worst at the Spitskop site

| Species | Provenance | State | Country | Spitskop (%) | Wilgeboom (%) |
|---|---|---|---|---|---|
| *P. tecunumanii* | *P. elliottii* control | Local source | South Africa | 83.3[A] | 100[A] |
| | *P. patula* control | Local source | South Africa | 69.4[AB] | 100[A] |
| | San Lorenso (HE) | Zacapa | Guatemala | 52.8 (2)[BCD] | 98.1(3)[AB] |
| | Montebello (HE) | Chiapas | Mexico | 52.4 (6)[CD] | 96.5 (8)[AB] |
| | San Jerónimo (HE) | Baja Verapaz | Guatemala | 45.0 (4)[CDE] | 93.7 (7)[ABC] |
| | Chempil (HE) | Chiapas | Mexico | 44.0 (9)[CE] | 94.0 (13)[ABC] |
| | Jitotol (HE) | Chiapas | Mexico | 41.7 (3)[CEF] | 88.0 (3)[CD] |
| | San José (HE) | Chiapas | Mexico | 36.1 (2)[EFG] | 87.5 (2)[CD] |
| | Las Trancas (HE) | La Paz | Honduras | 34.7 (2)[EFG] | 88.9 (3)[AC] |
| | Jocon (LE) | Yoro | Honduras | 30.6 (2)[FGH] | 86.8 (4)[D] |
| | San Esteban (LE) | Olancho | Honduras | 26.7 (9)[GH] | 85.3 (13)[D] |
| | San Francisco (LE) | Olancho | Honduras | 26.1 (15)[GH] | 83.6 (18)[D] |
| | Villa Santa (LE) | El Paraiso | Honduras | 22.9 (26)[D] | 85.7 (33)[D] |
| *Pinus maximinoi* | *P. elliottii* control | Local source | South Africa | 97.2[B] | 94.4[A] |
| | *P. patula* control | Local source | South Africa | 88.9[B] | 97.2[A] |
| | Marcala | La Paz | Honduras | 27.8 (2)[A] | 93.5 (3)[A] |
| | La Canada | Chiapas | Mexico | 23.6 (2)[A] | 86.1 (2)[A] |
| | Tatumbla | Fco. Morazán | Honduras | 21.3 (3)[A] | 88.9 (3)[A] |
| | San Juan Sacatepéquez | Guatemala | Guatemala | 19.4 (5)[A] | 86.1 (6)[A] |
| | San Jerónimo | Chiapas | Mexico | 17.9 (4)[A] | 85.3 (4)[A] |
| | San Jerónimo | Baja Verapaz | Guatemala | 17.4 (9)[A] | 86.8 (10)[A] |
| | El Portillo | Ocotepeque | Honduras | 16.7 (3)[A] | 87.0 (3)[A] |
| | Coban | Alta Verapaz | Guatemala | 13.3 (5)[A] | 83.8 (6)[A] |

Figures in brackets are the number of families representing each provenance

Treatments with different letters, for each species separately, are significantly different ($p < 0.05$)

The correlation between *P. tecunumanii* families was stronger ($r = 0.52$) than *P. maximinoi* ($r = 0.37$) (Figs. 4, 5).

## Discussion

This study provides quantitative evidence that frost tolerance is under genetic control in *P. tecunumanii* and *P. maximinoi,* which is similar to that observed in other pine species (Rehfeldt 1989; Duncan et al. 1996; Howe 2006). A substantial amount of the observed family variance in the selected population for *P. tecunumanii* appears related to the original country and provenance origin. These provenance effects were consistent across two distinct environments that were widely different in their frost survival means.
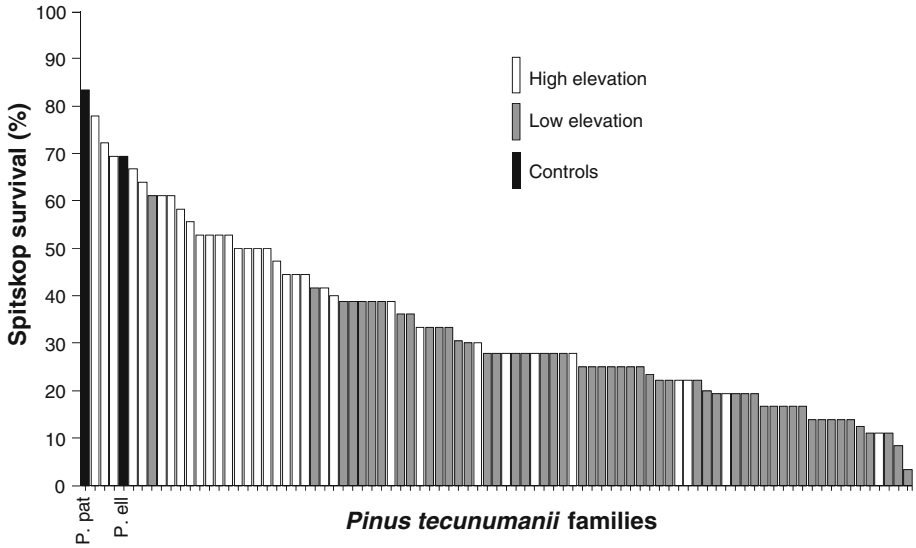
**Fig. 2** *P. tecunumanii* family survival on the cool-temperate site (Spitskop) ($h^2_{(0,1)} = 0.16$, $h^2_L = 0.27$)
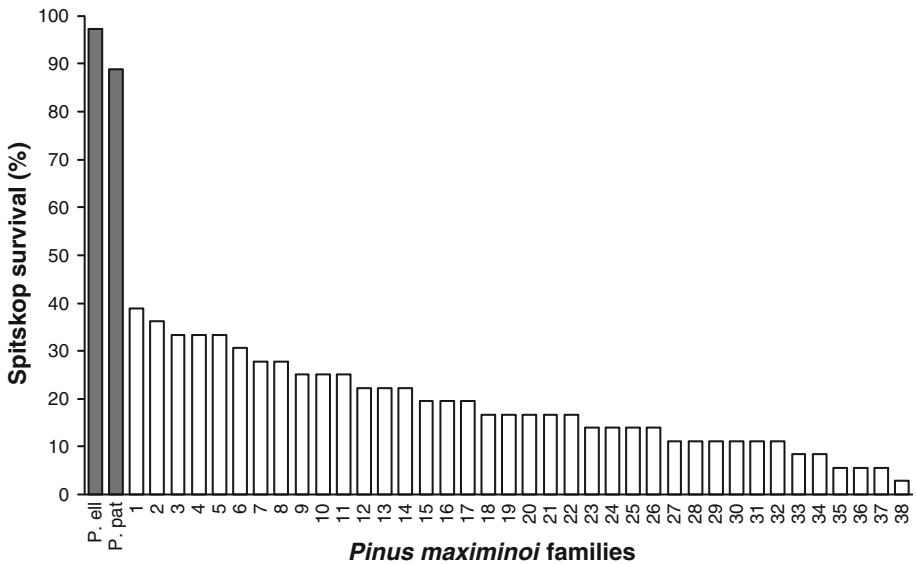


**Fig. 3** *P. maximinoi* family survival on the cool-temperate site (Spitskop) ($h^2_{(0,1)} = 0.11$, $h^2_L = 0.23$)

In this study, frost tolerance was measured using a binomial trait. Survival for both species on both sites was near the low or high end of the scale (19% for *P. maximinoi* and 33% for *P. tecunumanii* at Spitskop, and 86% for both species at Wilgeboom). Despite there being no significant differences between *P. maximinoi* provenances (Table 4), the ranking in survival on the two sites were very similar. Heritability estimates on the binomial scale were rather low, but on the underlying liability scale, $h^2_L$ estimates of 0.23 to

**Table 5** Heritability estimates for *Pinus maximinoi* and *P. tecunumanii* at the two sites

| Model | Site | Species | $H^2_{(0,1)}$ | SE | $H^2_{(L)}$ | SE |
|---|---|---|---|---|---|---|
| Family | Spitskop | *P. maximinoi* | 0.11 | 0.04 | 0.23 | 0.09 |
| Family | Spitskop | *P. tecunumanii* | 0.16 | 0.06 | 0.27 | 0.10 |
| Family | Wilgeboom | *P. maximinoi* | 0.00 | 0.00 | 0.00 | 0.00 |
| Family | Wilgeboom | *P. tecunumanii* | 0.05 | 0.03 | 0.12 | 0.07 |



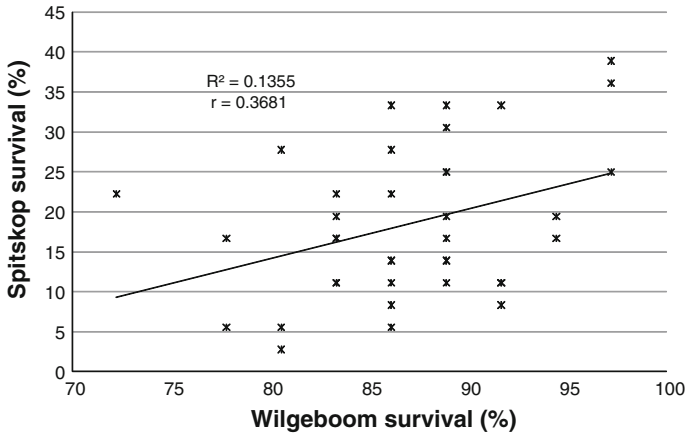**Fig. 4** The correlation between all *P. tecunumanii* families at the two sites



**Fig. 5** The correlation between all *P. maximinoi* families at the two sites

0.27 for the two species at the Spitskop site indicates that frost tolerance in both species could be improved through breeding and selection. *Pinus maximinoi* appeared to be more susceptible to cold damage than *P. tecunumanii*. Since breeding would begin with a lower mean tolerance in the population, it may require multiple generations to make any

significant improvement in the frost tolerance of this species. Compared to *P. maximinoi*, the broader range of family tolerance in *P. tecunumanii* (particularly of the high-elevation subgroup) and slightly higher heritability suggests that advances in breeding for frost tolerance in *P. tecunumanii* would be more easily achieved.

The *P. tecunumanii* high elevation provenances, Montebello, San Jerónimo and Chempil, which ranked as some of the more frost tolerant provenances in this study, have been found to be significantly more susceptible to *F. circinatum* than the high elevation provenances of Jitotol and Las Trancas (Mitchell et al. 2011), which were less tolerant of frost. Similarly, Villa Santa, which was significantly more frost susceptible compared to other low elevation provenances in this study, ranked as the most tolerant provenance to *F. circinatum* (Mitchell et al. 2011). This suggests that there is an inverse relationship between frost tolerance and tolerance to *F. circinatum* within *P. tecunumanii*. This means that selecting and breeding for frost tolerance in *P. tecunumanii*, and increasing the distribution of more frost tolerant selections to cool sites as a replacement for *P. patula*, may be limited by a decline in tolerance to *F. circinatum*.

Subsequent to the frost event in 2007, both species were replanted in February 2008 at the Wilgeboom site and *P. tecunumanii* was replanted at the Spitskop site in the same month. The survival was excellent at both sites. This illustrates that planting these species in a warmer month, followed by a normal winter period, can be successful. However, from our experience planting these species on sites, where frequent frost events are a normal occurrence and later than February in South Africa, should be avoided until their frost tolerance can be improved.

It is known that hybridizing frost-susceptible with tolerant species can provide an effective means to improve frost tolerance (Duncan et al. 1996). Therefore, to compliment breeding for frost tolerance, *P. tecunumanii* and *P. maximinoi* could be hybridized with tolerant species (such as *P. patula*). In such cases, whilst an improvement in frost tolerance can be seen, the frost tolerance of the hybrid may more closely resemble the susceptible parent (Duncan et al. 1996). This may be the experience in South Africa, where the *P. patula x P. tecunumanii* hybrid has become very popular due to its improved tolerance to *F. circinatum* (Roux et al. 2007), it remains susceptible to frost especially when the low elevation subgroup is used as the pollen parent. It is likely, however, that the susceptibility of *P. patula* × *P. tecunumanii* to frost can be improved upon by backcrossing it with *P. patula* as reported for other species (López-Upton et al. 1999). Importantly, the tolerance of hybrid families to frost seems more reliant on the specific combining ability of the parents and not necessarily the tolerance of the parents themselves (Duncan et al. 1996). Therefore, it seems likely that families of *P. patula* × *P. tecunumanii*, and not only the parents, would need to be tested for frost tolerance as well as for tolerance to *F. circinatum* in the future. This would also be the case where *P. maximinoi* is hybridized with other frost tolerant species.

As seen in this study, and elsewhere (López-Upton et al. 1999, Howe 2006, Dong et al. 2009), exposing young trees to cold temperatures in field trials may be an effective method to identify frost tolerant individuals. Based on our experience, temperatures slightly below freezing, for several hours per day for several days in the field should be sufficient to screen families for tolerance in *P. tecunumanii* and *P. maximinoi* in the field. However, a number of artificial screening methods have also been described (Rehfeldt 1989; South et al. 1993; Tinus et al. 2002; Mahalovich et al. 2006; Aldrete et al. 2008). Artificial tests using either seedlings/cuttings or needles, can be subjected to freezing temperatures in a controlled environment. After thawing, needles can simply be assessed for discoloration and bending ability and then the amount of damage scored (Rehfeldt 1989; South et al. 1993).

Alternatively the amount of electrolyte leakage from the damaged tissue can be scored (South et al. 1993; Tinus et al. 2002; Mahalovich et al. 2006; Aldrete et al. 2008). The results of these artificial tests often compare well with observations in the field (Howe 2006; Dvorak pers. comm. 2010). Whichever method is chosen to identify tolerant provenances and individuals, it will become increasingly important to improve the tolerance of subtropical species, such as *P. tecunumanii* and *P. maximinoi* to cold temperatures in South Africa.

## Conclusions and outlook

There was good evidence from this study that the frost susceptibility of *P. tecunumanii* and *P. maximinoi* is under genetic control, and can be improved by selecting provenances and families that are more tolerant to frost. Our hope is that through selection, we can eventually build up a sizeable population of individual trees with good frost tolerance and good growth. In order to maximize the potential that *P. tecunumanii* and *P. maximinoi* offer, particularly to reduce the risk posed by *F. circinatum*, frost tolerance will have to be included as a future selection criterion. Results of this study show that his can be achieved by planting provenances and families in cold climates and then recording mortality. Alternatively, various laboratory techniques should be explored to rapidly screen provenance and families that are most cold hardy. These can then be more thoroughly tested in the field under natural climatic conditions.

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