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Relative susceptibility of northern and southern provenances of *Pinus greggii* to infection by *Sphaeropsis sapinea*

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Abstract

Sphaeropsis sapinea is a well-known pathogen of *Pinus* spp. that causes severe die-back, in South Africa. In this study, 65 families representing both the northern and southern populations of *P. greggii* were evaluated for their tolerance to infection and subsequent die-back caused by *S. sapinea*. Families were evaluated for tolerance following natural infection after hail damage, as well as through inoculation. Variation in tolerance of trees after natural infections, occurred amongst families of both the northern and southern provenances, but highly significant differences in tolerance were observed between the two provenances. *P. greggii* trees of the southern provenances were significantly more susceptible to natural infection after hail damage. Artificial infection was not suitable for prediction of susceptibility to natural infection after hail damage. These observations have significant practical implications for plantation establishment in South Africa. © 2002 Elsevier Science B.V. All rights reserved.

1. Introduction

The search for *Pinus* species that are able to grow well on marginal sites and thus to sustain the development of commercial plantation forestry (Dvorak et al., 1996), has recently focused on species such as *P. greggii* Engelm. *P. greggii* is a member of the closed-cone pine group and occurs in two distinctly separate locations in central and northern Mexico between latitudes 20 and 26°N (Dvorak et al., 1996). The central or southern distribution of *P. greggii* is variable and includes environments ranging from those with high rainfall (1600 mm annually) and good soils to sites with rocky shallow soils and low rainfall (800 mm annually). These areas overlap with the distribution range of the closely related *P. patula* Schl. & Cham. In contrast, the northern distribution of *P. greggii* is much less variable and is generally drier (650 mm annual precipitation) and colder (Dvorak et al., 1996). *Pinus* species growing at high altitude (1200–2800 m) and in areas with low annual precipitation, are most desirable for commercial planting in marginal areas with elevated sites in the tropics and subtropics. This has, consequently, led to the establishment of *P. greggii* provenance trials in South Africa, Colombia and Brazil by the Central American and Mexico Coniferous Resources Co-operative (CAMCORE) (Dvorak et al., 1996).

Provenance trials of *P. greggii* established by CAMCORE have incorporated seed collections from

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the most complete distribution of this pine species in natural stands. These include a wide spectrum of genetic variability within the species. Results from these trials have indicated significant differences in growth rates (Dvorak et al., 1996), morphology (Donahue and Lopez-Upton, 1996) and terpene composition (Donahue et al., 1997). The hypothesis that the northern and southern provenances of *P. greggii* might represent different species has, however, not been fully tested and further genetic studies are required to consider this question completely.

A *P. greggii* provenance trial was established on the farm In de Diepte, Mpumalanga Province, South Africa (25°S, 1200 mm annual precipitation, 2150 m altitude) in 1992. During December 1995, trees in this trial were severely damaged by hail. The hail, damaged the trees represented in the trial uniformly, and extensive die-back was evident as early as March 1996. *Sphaeropsis sapinea* (Fr.:Fr.) Dyko and Sutton was found to be the causal agent of die-back symptoms observed on all pine species present in the trial.

S. sapinea is the most important pine pathogen in South Africa and causes extensive die-back of *P. patula* and *P. radiata* D. Don (Swart et al., 1987a,b; Zwolinski et al., 1990a,b) after hail damage. *P. elliottii* and *P. taeda* are relatively tolerant to infection and subsequent die-back caused by *S. sapinea* (Slagg and Wright, 1943; Bega et al., 1978; Swart et al., 1988). Dvorak et al. (1996) noted that *P. greggii* appeared to be more tolerant to post-hail associated die-back, caused by *S. sapinea*, than was *P. patula*, but did not elaborate on this hypothesis.

The aim of this study was to assess differences in relative susceptibility of the southern and northern provenances of *P. greggii* to post-hail associated die-back caused by *S. sapinea*. Comparisons were made with *P. patula*, which is known to be highly susceptible and *P. elliottii* and *P. taeda* that are resistant to infection by *S. sapinea*. Branch inoculations were also used as a measure of predicting susceptibility or tolerance of the pine species to infection and thus post-hail associated die-back caused by *S. sapinea*.

2. Materials and methods

2.1. Experimental layout

The trial was planted in a compact family design with open-pollinated families from each provenance grouped together within each of four replications. Six-tree plots in rows represented each family in every replication. Spacing between trees was 3.0 m × 3.0 m. The trial included 13 families from the southern and 51 families from the northern provenances of *P. greggii* and controls of *P. patula* (three seed lots), *P. elliottii* Engelm. (one seed lot) and *P. taeda* L. (two seed lots). The southern provenances originated from Laguna Seca (six families) and Laguna Atezca (one family) in Hidalgo and El Madroño (six families) in Queretaro. The northern provenances originated from La Tapona (18 families), Ojo de Agua (16 families), Las Placetas (2 families) and Cerro El Potosi (2 families) in Nuewo Leon and Jamé (7 families) and Cañon Los Lirios (6 families) in Coahuila (Table 1).

Table 1
Location of provenances of *P. greggii* in Mexico used in this study

Provenances	Latitude–longitude[a]	Elevation (m)[a]
Southern region		
Laguna Seca, Hidalgo	21°02′N–99°10′W	1670–1830
El Madroño, Queretaro	21°16′N–99°10′W	1650–1730
Laguna Atezca, Hidalgo	20°49′N–98°46′W	1250–1420
Northern region		
Jamé, Coahuila	25°21′N–100°37′W	2500–2590
Las Placetas, Nuevo Leon	24°55′N–100°11′W	2370–2520
Cañon Los Lirios, Coahuila	25°22′N–100°29′W	2260–2460
Cerro El Potosi, Nuevo Leon	24°54′N–100°12′W	2430–2500
Ojo de Agua, Nuevo Leon	24°53′N–100°13′W	2115–2400
La Tapona, Nuevo Leon	24°43′N–100°10′W	2090–2350

[a] Dvorak et al., 1996.

2.2. Natural die-back

The extent of natural die-back of all pine species represented in the trial was measured, three months after the hail damage, during March 1996. At this time, trees started to regenerate, thus assuming the start of re-growth and the end of active die-back. Die-back was measured as the difference between tree height and the height of the first living branch. Measurements were taken of all trees in the trial and to the nearest 0.01 m.

Isolations were made by dissecting tissue pieces from lesion margins of branches and stems that showed die-back symptoms (50 randomly selected trees from trial site). These tissue pieces were placed on 2% malt extract agar (MEA, Biolab) in petri dishes and incubated at 25 °C for up to 2 weeks. Dark-grey to black isolates were transferred to water agar with sterile pine needles (WA, Biolab). Pycnidia of *S. sapinea* formed on needles and the fungus was recovered from more than 90% of tissue pieces sampled in this way.

2.3. Inoculations

Replication one of the provenance trial was used in inoculation studies. Six trees, from each of the families of *P. greggii* as well as controls on *P. patula*, *P. elliottii* and *P. taeda*, were inoculated with an isolate of *S. sapinea* (CMW 1184, PREM 48859) that had previously been shown to be virulent in pathogenicity tests. The isolate was grown on 2% MEA in petri dishes for 5 days prior to inoculation. Inoculations were made during March 1996 (Autumn) and repeated in September 1996 (Spring). Inoculations were done on the undamaged bark of branches (approximately 3 cm diameter) minimally damaged by the hail. One branch was inoculated per tree. A cork borer (8 mm diameter) was used to remove bark discs. Bark discs were replaced by agar discs of the same size on which *S. sapinea* was growing. The wounds were sealed with masking tape to restrict desiccation. The lengths of the lesions that had formed in the cambium were measured after 4 weeks.

2.4. Statistical analysis

Analyses of variance were computed for the height of natural die-back and lesion length. Differences amongst families and within provenances were tested for significance using Tukey's procedure for the comparison of means ($P \leq 0.05$). The mean susceptibility of provenances of *P. greggii* was compared by contrast testing with F-distribution ($P \leq 0.05$) (Mead and Curnow, 1983). The effectiveness of using inoculations to predict susceptibility in pines to post-hail associated die-back was tested by using Spearman's coefficient of rank correlation (Ostle and Malone, 1994).

3. Results

3.1. Natural die-back

The three southern provenances of *P. greggii* differed significantly from one another ($P \leq 0.05$) (Table 2), with trees from Laguna Atezca showing the largest degree of die-back (mean = 202.6 cm, $s^2 = 0.00$), followed by those of El Madroño (mean = 193.1 cm, $s^2 = 7.25$) and Laguna Seca (mean = 140.9 cm, $s^2 = 11.32$). There were no significant differences in natural die-back, caused by *S. sapinea*, amongst *P. greggii* families from the southern provenances of Laguna Atezca in Hidalgo and El Madroño in Queretaro (Table 2). Family 24 of the El Madroño provenance showed the most severe die-back of all families of the southern provenances (family mean = 222.1 cm, $s^2 = 32.45$) and family 102 of the Laguna Seca provenance was the least severely damaged (family mean = 108.9 cm, $s^2 = 20.04$). Significant differences occurred amongst families of the Laguna Seca provenance, with family 108 showing significantly more die-back than families 102 and 103 (Table 2). All southern provenances had significantly ($P \leq 0.05$) more die-back than the *P. taeda* (mean = 79.6 cm, $s^2 = 6.33$) and *P. elliottii* (mean = 12.7 cm, $s^2 = 0.00$) seed sources, included as positive controls for tolerance to *S. sapinea* infections. No significant difference ($P \leq 0.05$), however, existed between southern provenances and the *P. patula* seed sources (mean = 148.8, $s^2 = 6.33$), that were included as positive controls for susceptibility to *S. sapinea* infection.

The nine northern provenances of *P. greggii* did not differ significantly from one another in terms of die-back ($P \leq 0.05$) (Table 2). The provenance Cañon Los

Table 2
Mean die-back of families of *P. greggii* and controls, caused by *S. sapinea* after hail

Family	Provenance/selection	Average die-back (cm)[a]
24	El Madroño	222.1 a
35	Laguna Atezca	202.6 ab
9	El Madroño	198.2 ab
11	El Madroño	189.7 abc
7	El Madroño	186.4 abcd
16	El Madroño	185.7 abcd
108	Laguna Seca	180.8 abcde
12	El Madroño	176.3 abcdef
999	*P. patula*, second generation orchard	166.2 bcdef
105	Laguna Seca	156.7 bcdefg
997	*P. patula*	141.1 cdefgh
998	*P. patula*, first generation orchard	139.0 defgh
106	Laguna Seca	135.4 efgh
100	Laguna Seca	134.5 efgh
103	Laguna Seca	129.3 fgh
102	Laguna Seca	108.9 ghi
995	*P. taeda*, second generation mix	92.2 hij
996	*P. taeda*, Texas origin	66.9 ijk
151	La Tapona	66.1 ijkl
95	Jamé	50.0 jklm
92	Jamé	43.3 jklm
131	Ojo de Agua	43.3 jklm
163	La Tapona	41.4 klm
138	Ojo de Agua	40.8 klm
136	Ojo de Agua	39.4 klm
153	La Tapona	37.1 klm
147	La Tapona	34.3 klm
89	Cañon Los Lirios	34.2 klm
66	Las Placetas	34.0 klm
85	Cañon Los Lirios	33.0 klm
145	Ojo de Agua	31.9 klm
82	Cañon Los Lirios	31.0 klm
166	La Tapona	30.8 klm
93	Jamé	30.2 klm
79	Cañon Los Lirios	29.8 klm
81	Cañon Los Lirios	29.4 klm
148	La Tapona	28.9 klm
77	Cañon Los Lirios	28.2 klm
157	La Tapona	28.2 klm
119	Ojo de Agua	28.0 klm
143	Ojo de Agua	28.0 klm
126	Ojo de Agua	27.4 klm
128	Ojo de Agua	26.8 klm
98	Jamé	25.7 klm
48	Cerro El Potosi	24.2 klm
97	Jamé	24.0 klm
65	Las Placetas	23.7 klm
144	Ojo de Agua	20.3 klm
167	La Tapona	20.2 klm
150	La Tapona	20.2 klm
146	La Tapona	19.5 klm
121	Ojo de Agua	19.5 klm
127	Ojo de Agua	18.3 klm
123	Ojo de Agua	18.3 klm
155	La Tapona	18.2 klm
125	Ojo de Agua	16.9 lm
90	Jamé	16.3 m
154	La Tapona	16.0 m
91	Jamé	15.8 m
118	Ojo de Agua	15.0 m
169	La Tapona	13.8 m
152	La Tapona	13.2 m
994	*P. elliottii*, second generation orchard	12.7 m
156	La Tapona	12.0 m
47	Cerro El Potosi	11.9 m
164	La Tapona	10.8 m
142	Ojo de Agua	10.6 m
149	La Tapona	10.1 m
135	Ojo de Agua	6.4 m
159	La Tapona	5.6 m

[a] Means in a column followed by the same letters are not significantly different from each other ($P \leq 0.05$).

Lirios showed the highest degree of die-back (mean $= 30.9$ cm, $s^2 = 1.03$) followed by Jamé (mean $= 29.3$ cm, $s^2 = 6.03$), Las Placetas (mean $= 28.9$ cm, $s^2 = 2.59$), Ojo de Agua (mean $= 24.4$ cm, $s^2 = 5.19$), La Tapona (mean $= 23.3$ cm, $s^2 = 7.13$) and Cerro El Potosi (mean $= 18.0$ cm, $s^2 = 3.06$). There were no significant differences in die-back amongst families belonging to the provenances of Las Placetas, Cerro El Potosi and Ojo de Agua from Nuevo Leon as well as Cañon Los Lirios from Coahuila. The northern provenance family that showed the most severe die back was family 151 from the La Tapona provenance (family mean $= 66.2$ cm, $s^2 = 16.90$) whereas family 135 from the Ojo de Agua provenance (family mean $= 6.4$ cm, $s^2 = 4.53$) showed the least die-back. The families with the most severe die-back were from the Jamé provenance (families 95 and 92) and the La Tapona provenance (families 151 and 163). Trees in these families did not exhibit significant differences from *P. taeda* seed sources, but showed significantly less die-back than the *P. patula* seed sources (Table 2). Die-back of trees of northern provenances of *P. greggii* did not differ significantly ($P \leq 0.05$) from those of the *P. elliottii* seed source, but showed significantly ($P \leq 0.05$) less die-back than both the *P. taeda* and

P. patula seed sources. Trees from northern provenances of *P. greggii* (overall mean = 21.9 cm, $s^2 = 11.28$) had significantly less die-back than trees of southern provenances (overall mean = 169.7 cm, $s^2 = 21.44$) after hail and subsequent *S. sapinea* infection.

3.2. Inoculations

No significant differences ($P \leq 0.05$) in lesion development were evident amongst families from the southern as well as families from the northern provenances of *P. greggii* and controls at either inoculation date (Table 3). The only exception was trees from the southern provenance Laguna Atezca that developed significantly larger lesions than all other provenances. This provenance, however, is comprised of only one family, that may explain the discrepancy. Northern provenances showed a tendency to produce smaller lesions, following inoculation with *S. sapinea*.

The low values of Spearman's ranking correlation coefficients (March inoculation $r_s = 0.318$; September inoculation $r_s = 0.252$) indicates that a weak relationship exists between the natural *S. sapinea* induced die-back following hail damage and lesion development following inoculation of branches. The fact that 90% (March inoculation) and 94% (September inoculation) of the differences observed between natural die-back and inoculations are unexplained (March inoculation $r_s^2 = 0.101$; September inoculation $r_s^2 = 0.064$), indicates that inoculation of branches is not a good parameter in the prediction of the tolerance of pine species, provenances or families to infection by *S. sapinea* following hail damage. This point may be best illustrated by the ranking discrepancies of the *P. elliottii* trees that were included for comparison due to their known tolerance to *S. sapinea* infection. Amongst the 70 families of *P. greggii*, *P. elliottii*, *P. taeda* and *P. patula* included in this trial, *P. elliottii* were ranked 61st on a scale of tolerance to natural die-back (1st = most susceptible). In contrast, it was ranked 7th (March inoculation) and 11th (September inoculation), respectively on a scale of lesion development in inoculation trials (1st = largest lesion developed).

Table 3
Means of natural die-back (cm) and lesion length (mm) observed in *P. greggii* provenances from the southern and northern regions, as well as control species

Provenance	Natural die-back[a]	Inoculation dates[a]	
		March	September
Southern region			
Laguna Atezca	202.6 a	21.7 a	15.5 a
El Madroño	193.1 b	17.1 b	15.0 a
Laguna Seca	140.6 c	14.2 b	23.5 a
Northern region			
Cañon Los Lirios	30.9 e	10.1 b	16.8 a
Jamé	29.3 e	7.2 b	14.8 a
Las Placetas	28.9 e	8.2 b	17.5 a
Ojo de Agua	24.4 e	6.1 b	13.2 a
La Tapona	23.7 e	6.4 b	13.7 a
Cerro El Potosi	18.0 e	13.0 b	17.9 a
Control species			
P. patula	148.8 c	17.5 b	21.7 a
P. taeda	79.6 d	12.4 b	18.4 a
P. elliottii	12.7 e	14.2 b	19.5 a

[a] Means in columns followed by the same letter do not differ significantly from each other (contrast testing $P \leq 0.05$). Contrast testing were used to compare provenances with one another despite the different number of families contributing to each provenance.

4. Conclusions

Trees of *P. greggii* representing the provenances from central (southern) and northern Mexico could easily be distinguished from one another based on their susceptibility to *S. sapinea* infection after hail. Trees of the southern provenances were significantly more susceptible to infection by the pathogen and subsequently displayed more dramatic die-back than those of the northern provenances. The differences in *S. sapinea* associated die-back observed between the southern and northern provenances of *P. greggii* are perhaps not surprising given the fact that these trees differ notably in morphology (Donahue and Lopez-Upton, 1996), growth (Dvorak et al., 1996) and terpene chemistry (Donahue et al., 1997).

The susceptibility of southern as opposed to northern provenances of *P. greggii* to *S. sapinea* infection after hail is an important characteristic, when selecting provenances with potential for commercial planting or hybridisation programmes in South Africa. Summer rainfall areas frequently have hail-storms that cause devastating losses due to die-back caused by *S. sapinea* (Swart et al., 1987a,b, Zwolinski et al., 1990a,b).

S. sapinea is the most important pathogen of pines in South Africa. It is, thus, an important discovery that some forms of *P. greggii* are tolerant to this pathogen, and this will have important implications for the forestry industry.

P. greggii is highly desirable due to its tolerance to altitude and drought. It is, thus, a major advantage that this species can hybridise with *P. patula*, the most important *Pinus* sp. planted in South Africa. There is, thus, an opportunity to breed for drought tolerance and high altitude adaptation in *P. patula*, through hybridisation with *P. greggii*. The problem, however, is that *P. patula* is very susceptible to *S. sapinea* infection. In our opinion there is tremendous potential for hybridisation of *P. patula* and the northern provenances of *P. greggii* to obtain *S. sapinea* tolerance.

Using inoculation studies, we were able to determine the relative susceptibility of northern and southern provenances of *P. greggii* to *S. sapinea*. Northern provenances were not significantly more tolerant to inoculations with *S. sapinea* but showed a tendency to form smaller lesions, indicating similarities with die-back after hail. This technique could, however, not distinguish the tolerance of different families within provenance. The failure of this technique to predict response of different families under natural conditions is amply illustrated by the low Spearman's ranking coefficients obtained. This insensitivity may be reduced by increasing the period from inoculation to lesion measurement to more than 4 weeks. *P. elliottii* was extremely tolerant to natural post-hail associated die-back but showed significant lesion development after artificial inoculation. *P. elliottii* is widely regarded to be tolerant to infection by *S. sapinea* (Slagg and Wright, 1943; Bega et al., 1978; Swart et al., 1988). The reason why this species displayed susceptibility in these trees is unclear and deserves further study.

There exists a strong possibility that the use of the northern provenances of *P. greggii* in hybridisation programs with *P. patula*, will lead to increases in tolerance to *S. sapinea* infection. This, however, will be a slow process and the material produced will need to be thoroughly tested under field conditions before definitive predictions can be made. The results obtained in this study may, however, vary as a result of intraspecific variation with *S. sapinea* (Swart et al., 1987b) populations from different parts of the world.

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