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Variation in pitch canker resistance among provenances of *Pinus patula* and *Pinus tecunumanii* from Mexico and Central America

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Abstract *Pinus patula* and high-elevation (HE) sources of *P. tecunumanii* exhibit intermediate levels of resistance to pitch canker (*Fusarium circinatum*), compared to extremely resistant species such as *P. oocarpa*, and extremely susceptible species such as *P. radiata*. Seedlings from 20 *P. patula* provenances and 15 HE *P. tecunumanii* provenances were artificially inoculated with the pitch canker fungus at 21 and 12 weeks of age, respectively, and assessed for resistance 12–20 weeks later. There was important provenance variation in pitch canker resistance for both species. The 20-week LiveStem percentage ranged from 70.3% to 43.6% among the *P. patula* provenances and 59.6% to 11.7% among HE *P. tecunumanii* provenances. There was a geographic pattern to the provenance variation, and in both species, low altitude sources demonstrated more resistance than those from high elevation. Provenance variation in pitch canker resistance could be useful when making selection and breeding decisions with these species.

Keywords Tree diseases · Provenance variation · Artificial inoculation

Introduction

Pitch canker is a disease of pines, which is caused by the pathogen *Fusarium circinatum* ex *Pinus* spp. (Nirenberg and O'Donnell 1998). The disease results in dieback of branch tips, formation of cankers on the main stem, and production of copious amounts of resin. The disease was first described in the southeastern US in the late 1940s (Hepting and Roth 1946), but the pathogen is also widely distributed in Mexico, having been identified on 19 pine species in 14 states (Guerra-Santos 1999). Under the worst-case scenario, an infected tree can eventually lose vigor and die. Pitch canker has never reached epidemic proportion in the southern US, and its

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severity varies year to year apparently depending on the degree of weather-related and mechanical damage to trees (Dwinell et al. 1985).

In the last two decades, the disease has been reported in natural stands of *P. radiata* in California where it has reached epidemic proportions (Gordon et al. 2001). It has also spread to *Pinus patula* (Vilojen et al. 1994) and *P. radiata* nurseries (J. Mather, personal communication) in South Africa and *P. radiata* nurseries in Chile (Wingfield et al. 2002). Together, both species account for 4.7 million hectares of exotic plantations world-wide (Balocchi 1997; Birks and Barnes 1991), and therefore control of the disease is of great importance to the wood and forest products industry.

In efforts to find pine species that might be resistant to pitch canker either as pure species or in hybrid combination, the Camcore program at North Carolina State University screened seedlings of 16 pine species in the Oocarpae (Mexican closed pines), Australes (Southern US & Caribbean pines), and Attenuatae (California closed-cone pines) subsections and Pseuduostrobus Group at the USDA US Forest Service Resistance Screening Center (RSC) in Bent Creek, NC (Hodge and Dvorak 2000). The results indicated that the some species like *Pinus oocarpa, P. jaliscana*, and *P. pringlei* were very resistant to the disease, while others like *P. patula* and *P. greggii* showed extreme susceptibility. Interestingly, the susceptibility of *P. tecunumanii* in the Oocarpae varied considerably. Seedlings grown from seed samples of *P. tecunumanii* taken below 1,500 m altitude in Mexico and Central America (low elevation (LE) sources) were very resistant, while those sampled above 1,500 m altitude (high elevation (HE) sources) were only moderately resistant.

Even though there is now some information about the susceptibility of various pine species to pitch canker, little is known about provenance variation in susceptibility to the disease and the potential for developing resistant plantations through provenance selection and breeding. The objectives of this study were to examine provenance variation in pitch canker resistance for both *P. patula* and *P. tecunumanii* (HE), and to offer explanations why some populations in Mexico and Central America might be more susceptible than others.

Materials and methods

The 20 *P. patula* provenances and the 15 *P. tecunumanii* (HE) provenances included in these studies are presented in Tables 1 and 2. In the original seed collections completed by Camcore, around 20 mother trees per provenance were sampled, with a goal of at least 100 m between selected trees. The bulk provenance mixtures were composed of open-pollinated seed from a minimum of 6 randomly selected families. In addition to these provenances, the *P. tecunumanii* (HE) experiment included bulk mixes of *P. oocarpa, P. maximinoi, P. patula*, and LE *P. tecunumanii.* A *P. elliottii* pitch-canker-susceptible seedlot (FA2) was also included in both experiments according to the standard protocol at the RSC.

All seedlings were subjected to the pitch canker resistance screening protocols as developed by Oak et al. (1987), in which seedlings are challenged with the pitch canker fungus and their resultant responses are used to gauge relative resistance to infection. The *P. patula* experiment was conducted in 2003, and the *P. tecunumanii* experiment was conducted in 2004, with the dates of all activities listed in Table 3. Seed of all taxa were soaked in cold water for 24 h prior to sowing, and seedlings

Table 1 *Pinus patula* provenances included in the pitch canker resistance study

Code	Provenance	Dept/State, Country	Lat	Long	Elev (m)	Precip (mm)
20	Calcahualco	Veracruz, Mexico	19°07′ N	97°06′ W	2,375	2,020
8	Conrado Castillo	Tamaulipas, Mexico	23°56′ N	99°28′ W	1,780	1,012
3	Corralitla	Veracruz, Mexico	18°38′ N	97°06′ W	2,115	2,500
19	Cruz Blanca	Veracruz, Mexico	19°39′ N	97°09′ W	2,500	1,347
18	Cumbre de Muridores	Hidalgo, Mexico	20°19′ N	98°21′ W	2,430	1,869
15	El Cielo	Tamaulipas, Mexico	23°04′ N	99°14′ W	1,665	1,200
4	El Manzanal	Oaxaca, Mexico	16°06′ N	96°33′ W	2,505	1,348
5	El Tlacuache	Oaxaca, Mexico	16°44′ N	97°09′ W	2,460	2,000
2	Ingenio del Rosario	Veracruz, Mexico	19°31′ N	97°06′ W	2,820	1,346
17	La Cruz	Hidalgo, Mexico	20°17′ N	98°18′ W	2,375	1,869
16	La Encarnación	Hidalgo, Mexico	20°53′ N	99°13′ W	2,525	1,200
13	Llano de las Carmonas	Puebla, Mexico	19°48’ N	97°54’ W	2,705	1,097
11	Pinal de Amoles	Querétaro, Mexico	21°07′ N	99°41′ W	2,465	1,350
1	Potrero de Monroy	Veracruz, Mexico	20°24′ N	98°25′ W	2,400	1,350
24	San Mateo Río Hondo	Oaxaca, Mexico	16°08′ N	96°29′ W	2,440	1,300
7	Santa María Papalo	Oaxaca, Mexico	17°49′ N	96°48′ W	2,495	1,100
22	Sierra Huayacocotla	Veracruz, Mexico	20°29′ N	98°28′ W	2,350	1,405
10	Tlacotla	Tlaxcala, Mexico	19°40′ N	98°05′ W	2,833	1,097
25	Yextla	Guerrero, Mexico	17°36′ N	99°51′ W	2,295	1,700
12	Zacualtipán	Hidalgo, Mexico	20°39′ N	98°40′ W	2,090	2,047

Table 2 *Pinus tecunumanii* (high-elevation) provenances included in the pitch canker resistance study

Code	Provenance	Dept/State, Country	Lat	Long	Elev (m)	Precip (mm)
21	Cabricán	Quetzaltenango, Guatemala	15°35′ N	91°38′ W	2,590	1,010
10	Chanal	Chiapas, Mexico	16°42′ N	92°23′ W	2,180	1,238
30	Chiquival Viejo	Quetzaltenango, Guatemala	15°08′ N	91°33′ W	2,300	975
27	El Ingenio	Jalapa, Guatemala	14°43′ N	90°02′ W	1,885	1,400
25	El Pinalón	El Progreso, Guatemala	15°04′ N	89°54′ W	2,435	2,592
24	La Piedad	El Progreso, Guatemala	15°02′ N	90°02′ W	2,155	2,592
15	Las Piedrecitas	Chiapas, Mexico	16°42′ N	92°35′ W	2,430	1,252
16	Montebello	Chiapas, Mexico	16°06′ N	91°45′ W	1,705	1,909
29	Montecristo	Santa Ana, El Salvador	14°25′ N	89°24′ W	1,775	1,997
17	Napite	Chiapas, Mexico	16°34′ N	92°19′ W	2,210	1,350
18	Rancho Nuevo	Chiapas, Mexico	16°41′ N	92°35′ W	2,310	1,238
28	Río Chiquito	Chalatenango, El Salvador	14°22′ N	89°08′ W	2,115	1,629
5	San Jerónimo	Baja Verapaz, Guatemala	15°03′ N	90°18′ W	1,735	1,200
6	San Lorenzo	Zacapa, Guatemala	15°05′ N	89°40′ W	2,000	1,700
7	San Vicente	Baja Verapaz, Guatemala	15°05′ N	90°07′ W	1,945	1,700

were grown in Ray Leach® containers (115 ml). Single spore isolates (i.e., a single genotype) were used to prepare four bulks of conidia of *Fusarium circinatum* according to McRae et al. (1987). The isolates originated in four locations in Georgia and Florida in the southeastern US (Table 4). The seedlings were wounded by severing the stem just below the apical meristem, and the excised apical portion was removed. The seedlings were then inoculated by atomizing an aqueous spore suspension onto the fresh wounds, with a concentration of 25,000 spores/ml for the *P. patula* study and 50,000 spores/ml for the *P. tecunumanii* study. The atomized spore suspension was sprayed directly onto the wound surface from a distance of around 25 cm, passing 3 times over each tree. Each family was represented by 80

Table 3 Calendar of sowing, inoculation, and damage assessment for pitch canker screening of provenances of *P. patula* and high-elevation *P. tecunumanii*

Activity	*Pinus patula*		*Pinus tecunumanii*	
	Date	Timespan	Date	Timespan
Sowing	March 25, 2003		May 31, 2004	
Inoculation	August 18–20, 2003	21 weeks old	August 23–24, 2004	12 weeks old
First Damage Measurement	November 11, 2003	12 weeks post-inoculation	December 13, 2004	16 weeks post-inoculation
Second Damage Measurement	January 6, 2004	20 weeks post-inoculation	January 6, 2005	20 weeks post-inoculation

Table 4 Source of isolates used to screen provenances of *P. patula* and high-elevation *P. tecunumanii*

Isolate	Location	Host Species
S298	Gilchrist, FL	*P. elliottii*
S396	Bainbridge, GA	*P. taeda*
S397	Bainbridge, GA	*P. taeda*
S402	Milton, FL	*P. elliottii*

seedlings, with 20 seedlings in each of four replications. Each replication was inoculated with one of the four single-spore isolate mixtures.

All seedlings in the HE *P. tecunumanii* experiment were inoculated at age 12 weeks, and all seedlings in the *Pinus patula* experiment were inoculated at age 21 weeks. Previous work at the RSC with *P. patula* and *P. radiata* families has shown that better differentiation among families was obtained using older seedlings.

Following inoculation the seedlings were returned to the greenhouse where they were maintained for 20 weeks during which time pathogen colonization was allowed to occur. Two measurements of damage were taken in both experiments: 12 weeks and 20 weeks in the *P. patula* study, and 16 weeks and 20 weeks in the *P. tecunumanii* study. In both studies, the total length of the stem from hypocotyl to wound and total length of remaining live stem was measured at the first measurement date. At the second measurement date, the percent of live stem remaining was visually estimated to the nearest 10%. Two criteria were used to estimate damage at each measurement date:

$$\text{Dieback (mm)} = \text{absolute length of stem killed by the pathogen}$$

$$\text{LiveStem (\%)} = \text{percent of the original stem still alive at time of measurement}$$

Statistical methods

For each of the two damage traits in both experiments, ANOVA was conducted using SAS Proc GLM (SAS Institute 1989) using a linear model including seedling

height as a covariate, isolate-rep, provenance, and provenance × isolate-rep interaction. Since the primary objective of this study was to examine provenance variation, it was convenient to treat each 20-tree replication with a single isolate, thus isolates and replication were confounded. Previous experiments have demonstrated that replication effects are generally small, and therefore all parameters associated with isolate-rep effects were interpreted as being due mostly to isolate effects.

Variance components were estimated and Best Linear Unbiased predictions of provenance effects were calculated using SAS Proc Mixed. The proportion of provenance variance was estimated with the parameter p^2 estimated as

$$p^2 = \sigma^2_{\mathrm{p}}/(\sigma^2_{\mathrm{p}} + \sigma^2_{\mathrm{p\times t}} + \sigma^2_{\mathrm{e}}). \quad (1)$$

where σ^2_{p} = provenance variance, $\sigma^2_{\mathrm{p\times t}}$ = provenance × isolate-rep interaction variance, and σ^2_{e} = error variance. The Type B correlation of provenance effects across isolate-rep treatments was estimated as

$$r_{\mathrm{Bt}} = \sigma^2_{\mathrm{p}}/(\sigma^2_{\mathrm{p}} + \sigma^2_{\mathrm{p\times t}}). \quad (2)$$

This parameter is analogous to the Type B genetic correlation of Burdon (1977), and will approach 1 as the provenance × isolate-rep interaction variance approaches zero.

The repeatability of provenance means (R_{pm}) was estimated as

$$R_{\mathrm{pm}} = \sigma^2_{\mathrm{p}}/(\sigma^2_{\mathrm{p}} + \sigma^2_{\mathrm{p\times t}}/4 + \sigma^2_{\mathrm{e}}/80).$$

These parameter estimates were used to evaluate which trait gives the most precise discrimination among provenances.

Results

Height

There were statistically significant effects of seedling height on nearly all damage traits in both experiments (Tables 5 and 6). This effect was also observed in an experiment with 23 pine species/varieties (Hodge and Dvorak 2000). Presumably

Table 5 Mean damage on *P. patula* for the four inoculum-replication treatments measured 16 and 20 weeks after inoculation of 21-week-old seedlings

Inoc	Height (mm)	12-week damage measurement		20-week damage measurement	
		Dieback (mm)	LiveStem (%)	Dieback (mm)	LiveStem (%)
S298	258.8	61.4	74.5	129.6	47.2
S396	290.8	41.9	84.7	114.5	58.9
S397	237.7	50.2	77.5	114.5	49.8
S402	244.8	20.5	90.7	63.1	71.7
MEAN	258.0	43.5	81.9	105.4	56.9

Table 6 Mean damage on high-elevation *P. tecunumanii* for the four inoculum-replication treatments measured 16 and 20 weeks after inoculation of 12-week-old seedlings

Inoc	Height (mm)	16-week damage measurement		20-week damage measurement	
		Dieback (mm)	LiveStem (%)	Dieback (mm)	LiveStem (%)
S298	73.1	40.8	41.8	47.3	33.7
S396	80.9	45.3	41.1	53.6	31.2
S397	86.4	40.9	49.2	52.1	36.7
S402	79.0	44.5	41.1	52.2	32.4
MEAN	79.8	42.9	43.3	51.3	33.6

seedling height is a proxy for some type of physiological change, perhaps degree of lignification that impacts how quickly the pathogen can colonize the stem. All statistical analyses included height as a covariate.

Isolate effects

There were highly significant differences among isolate-reps for all damage traits at both measurements in the *P. patula* experiment (Table 7). Mean stem height was around 258 mm at the time of inoculation, and mean height in the isolate-reps ranged from 238 mm to 291 mm (Table 4). Isolate-reps were grown near one another, but in different areas of the greenhouse. Although height was used as a covariate, the height differences suggest the possibility of other physiological effects arising from the environmental differences among replications leading to moderate differences in pitch canker damage. Mean Dieback at 12 weeks post-inoculation ranged from 20.5 mm for isolate S402 to 61.4 mm for isolate S298 (Table 5). These values correspond to about 90.7% and 74.5% LiveStem, respectively. The differences persisted until the 20-week measurement, with mean Dieback at that time ranging from 63.1 mm for isolate S402 to 129.6 mm for isolate S298 (LiveStem of 71.7% and 47.2%, respectively).

In contrast to the *P. patula* experiment, in the *P. tecunumanii* experiment there were no significant differences among isolate-reps for any trait (Table 8).

Table 7 Summary of ANOVAs for pitch canker resistance traits in *P. patula* provenances[a]

Reading	Trait	Mean	*P*-value for significance[b]			
			Height	Isolate	Prov.	Prov. × isolate
12 weeks	Dieback	43.6 mm	*	****	****	****
	LiveStem	81.9 %	****	****	****	****
20 weeks	Dieback	105.4 mm	ns	****	****	****
	LiveStem	56.9 %	****	****	****	**

[a] Stem necrosis was measured 12 and 20 weeks after inoculation of 21-week old seedlings. Disease traits were analyzed using height as a covariate

[b] *P*-value < 0.0001 represented by ****

P-value < 0.001 represented by ***

P-value < 0.01 represented by **

P-value < 0.05 represented by *

Non-significant *P*-value represented by ns

Table 8 Summary of ANOVAs for pitch canker resistance traits in high-elevation *P. tecunumanii* provenances[a]

Reading	Trait	Mean	*P*-value for significance[b]			
			Height	Isolate	Prov.	Prov. × isolate
12 weeks	Dieback	42.9 mm	***	ns	****	ns
	LiveStem	43.2 %	****	ns	****	ns
20 weeks	Dieback	51.3 mm	****	ns	****	ns
	LiveStem	33.5 %	****	ns	****	ns

[a] Stem necrosis was measured 16 and 20 weeks after inoculation of 12-week old seedlings. Disease traits were analyzed using height as a covariate

[b] *P*-value < 0.0001 represented by ****

P-value < 0.001 represented by ***

P-value < 0.01 represented by **

P-value < 0.05 represented by *

Non-significant p-value represented by ns

Provenance × isolate-rep interaction

In the *P. patula* experiment, there were statistically significant provenance × isolate-rep interactions for all damage traits. The Type B provenance correlations ranged from 0.41 to 0.84 for the Dieback and LiveStem traits (Table 9), indicating a moderate amount of interaction across isolate-reps. Conversely, there was no significant provenance × isolate-rep interaction for any of the damage traits at either measurement for *P. tecunumanii* (Table 6). The Type B provenance correlations were uniformly high, ranging from 0.92 to 1.00 for all traits (Table 10), indicating no biologically important interaction with isolates.

Table 9 Genetic parameters[a] for pitch canker resistance traits for provenances of *P. patula* measured 12 and 20 weeks after inoculation. Disease traits analyzed using height as covariate

Trait	Mean	p^2	r_{Bt}	R_{pm}	σ^2_p	$\sigma^2_{p\times t}$	σ^2_e
Height	258.0 mm	0.112	0.63	0.81	477.9	277.6	3515.8
Dieback_12	43.6 mm	0.095	0.61	0.79	82.4	53.5	735.0
LiveStem_12	81.9%	0.030	0.41	0.57	31.8	45.1	1002.3
Dieback _20	105.4 mm	0.065	0.56	0.73	539.3	428.6	7380.1
LiveStem_20	56.9%	0.088	0.84	0.85	201.6	37.8	2057.7

[a] Genetic parameters are:

p^2 = the proportion of provenance variance

r_{Bt} = the Type B correlation of provenance effects across isolate-rep treatments

R_{pm} = the repeatability of provenance means

σ^2_p = provenance variance

$\sigma^2_{p\times t}$ = provenance × isolate-rep interaction variance, and

σ^2_e = error variance

Table 10 Genetic parameters[a] for pitch canker resistance traits for provenances of high-elevation *P. tecunumanii* measured 16 and 20 weeks after inoculation. Disease traits analyzed using height as covariate

Trait	Mean	p^2	r_{Bt}	R_{pm}	σ^2_p	$\sigma^2_{p\times t}$	σ^2_e
Height	79.8 mm	0.162	0.57	0.80	69.4	52.2	307.1
Dieback_16	42.9 mm	0.099	0.92	0.88	103.9	9.1	940.6
LiveStem_16	43.3%	0.115	0.98	0.91	183.1	3.5	1399.5
Dieback _20	51.3 mm	0.084	1.00	0.88	107.3	0.0	1166.7
LiveStem_20	33.5%	0.093	1.00	0.89	165.3	0.0	1613.4

[a] Genetic parameters are:

p^2 = the proportion of provenance variance

r_{Bt} = the Type B correlation of provenance effects across isolate-rep treatments

R_{pm} = the repeatability of provenance means

σ^2_p = provenance variance

$\sigma^2_{p\times t}$ = provenance × isolate-rep interaction variance, and

σ^2_e = error variance

Species and provenance differences

The species differences that were observed in an earlier study (Hodge and Dvorak 2000) are completely consistent with those seen in this study (Table 12). In terms of resistance, *P. oocarpa* > *LE P. tecunumanii* > *P. maximinoi* > *HE P. tecunumanii* > *P. patula.* In the *P. tecunumanii* experiment, seedlings of *Pinus oocarpa* inoculated at 12 weeks had 92.6% LiveStem 20 weeks after inoculation, while LE *P. tecunumanii* was nearly as resistant, with 90.2% mean LiveStem. On average, HE *P. tecunumanii* averaged 33.5% LiveStem, while *P. patula* was almost completely dead, with mean LiveStem only 2.3%. It is difficult to make precise comparisons among the two experiments due to the difference in age of inoculation in the two experiments (21 weeks for the *P. patula* study, and 12 weeks for the *P. tecunumanii* study), however the following statements seem reasonable:

- the most resistant *P. patula* provenances might approach the mean resistance of HE *P. tecunumanii*,
- the most resistant HE *P. tecunumanii* provenances might approach the mean resistance of *P. maximinoi*, but would still be less resistant than typical LE *P. tecunumanii*.

The most useful trait to discriminate among provenances in the *P. patula* experiment was 20-week LiveStem, with R_{pm} = 0.85 (repeatability of provenance means, Table 9). In the *P. tecunumanii* experiment, there was little difference among traits for R_{pm}, with values ranging from 0.88 to 0.91 (Table 10). As one might expect, there were no important differences in how HE *P. tecunumanii* provenances ranked according to the different damage traits.

There were significant and important differences among provenances for all damage traits in both the *P. patula* and the *P. tecunumanii* experiments (Table 5 and 6). Using 20-week LiveStem to rank the *P. patula* provenances, the most resistant provenance (El Cielo) had 70.3% LiveStem, while the most susceptible provenance (Corralitla) had only 43.6% LiveStem (Table 11). In the *P. tecunumanii* experiment,

Table 11 Provenance ranking for pitch canker resistance of *P. patula* provenances measured 12 and 20 weeks after inoculation of 21-week-old seedlings[a]

Provenance	Code	Elev (m)	ht (mm)	12-week measurement		20-week measurement	
				Dieback (mm)	LiveStem (%)	Dieback (mm)	LiveStem (%)
El Cielo	15	1665	258.1	32.8	86.4	73.0	70.3
Yextla	25	2295	231.0	29.2	88.8	80.5	69.3
Conrado Castillo	8	1780	282.6	32.7	85.9	74.1	68.2
El Tlacuache	5	2460	224.1	33.7	86.4	83.2	67.5
La Encarnación	16	2525	272.2	34.0	85.5	87.5	64.0
Pinal Amoles	11	2465	267.8	37.7	83.5	88.0	62.6
Potrero de Monroy	1	2400	272.1	41.2	83.0	100.3	59.9
Tlacotla	10	2833	233.5	42.7	83.1	103.0	58.8
Zacualtipán	12	2090	270.4	43.5	80.6	100.1	57.5
Mean *P. patula*			258.0	43.5	81.9	105.4	56.9
Sierra Huayacocotla	22	2350	261.5	43.7	80.8	103.4	55.0
Calcahualco	20	2375	256.3	46.6	80.1	107.4	54.4
Santa María Papalo	7	2495	255.3	49.6	80.1	115.6	53.9
Cumbre de Muridores	18	2430	255.5	47.0	79.9	112.1	53.3
San Mateo	24	2440	239.5	43.8	82.3	116.5	52.7
P. elliottii (susc. FA2)			208.0	44.3	78.5	99.7	52.2
El Manzanal	4	2505	229.2	47.2	80.4	116.7	51.4
La Cruz	17	2375	283.3	48.4	80.3	123.6	50.7
Ingenio de Rosario	2	2820	274.4	54.7	77.8	125.3	50.4
Llano de las Carmonas	13	2705	242.8	53.2	77.9	123.9	49.1
Cruz Blanca / Manzanillas	19	2500	258.6	54.1	77.9	137.4	45.4
Corralitla	3	2115	291.9	54.3	77.4	136.4	43.6

[a] Provenances are ordered by 20-week LiveStem. Values for *P. patula* provenances are best linear unbiased predictions using 21-week-old seedling height as covariate, values for *P. elliottii* control are unadjusted means

20-week LiveStem ranged from 59.6% for Montecristo to 11.7% for El Pinalón (Table 12). The provenance differences observed suggest that there is potential to improve pitch canker resistance in both taxonomic groups through selection and breeding. In both species, provenance differences were correlated with elevation, with lower collection sites demonstrating more resistance. For HE *P. tecunumanii*, the correlation between 20-week LiveStem and elevation was 0.58 (significant at $\alpha = 0.05$), while in *P. patula* this correlation was 0.45 (significant at $\alpha = 0.05$). In *P. patula*, there was also a moderately strong correlation between 20-week LiveStem and longitude ($R = -0.70$, significant at $\alpha = 0.001$), with more westerly provenances demonstrating more resistance.

Discussion

Pinus patula and *P. tecunumanii* can easily be separated morphologically (Perry 1991) and by RAPD molecular markers (Grattapaglia et al. 1992; Furman et al. 1997, Dvorak et al. 2001). Both species appear to have evolved from an ancient *P. oocarpa* progenitor (Dvorak et al. 2000). The separation of *P. tecunumanii* into HE and LE groups was originally based on subtle morphological differences in bark

Table 12 Provenance ranking for pitch canker resistance of high-elevation *P. tecunumanii* provenances measured 16 and 20 weeks after inoculation of 12-week-old seedlings[a]

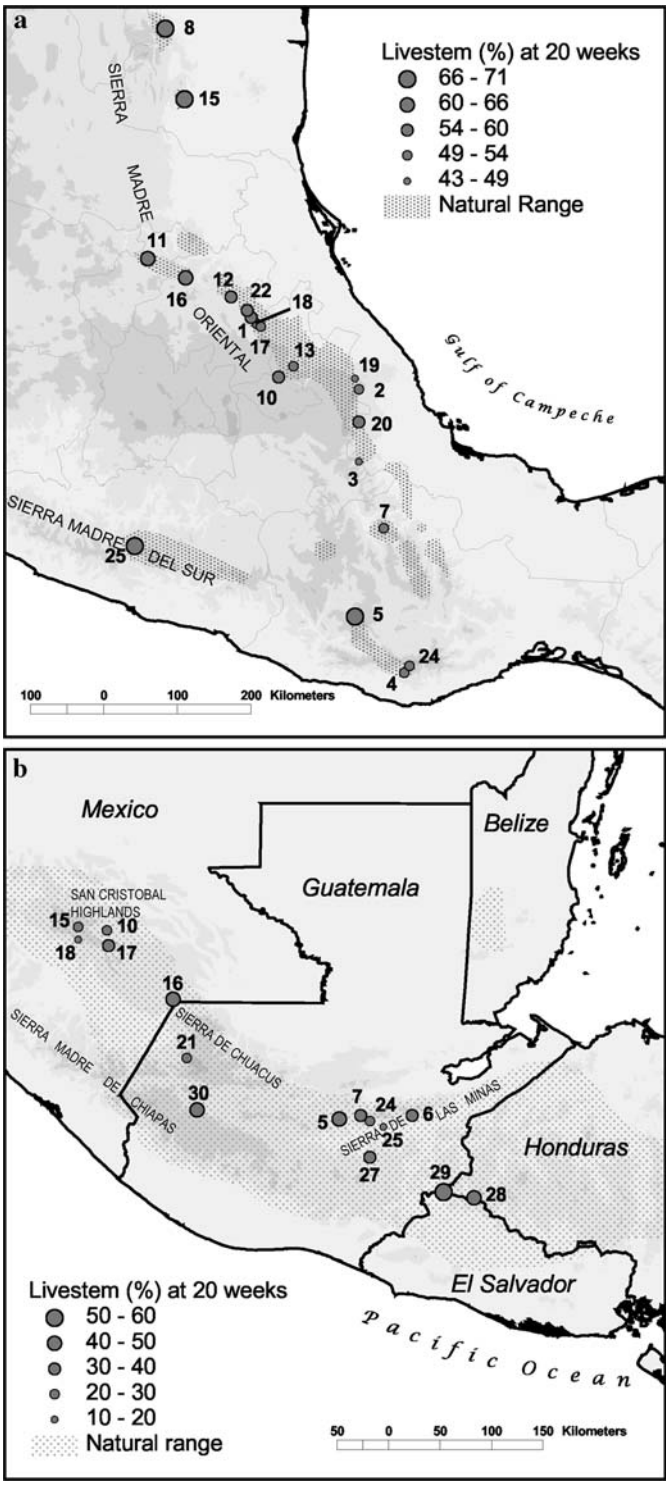
Provenance	Code	Elev (m)	ht (mm)	16-week measurement		20-week measurement	
				Dieback (mm)	LiveStem (%)	Dieback (mm)	LiveStem (%)
P. oocarpa			126.1	6.1	95.1	9.4	92.6
P. tecunumanii (LE)			111.4	8.9	92.0	11.0	90.2
P. maximinoi			111.6	21.6	79.7	30.6	71.7
Montecristo	29	1775	82.9	24.1	68.0	29.8	59.6
Río Chiquito	28	2115	83.4	35.2	53.4	42.2	44.2
Montebello	16	1705	85.4	40.6	49.7	45.6	43.3
Chiquival Viejo	30	2300	93.9	31.8	54.0	41.2	42.4
San Jerónimo	5	1735	78.3	38.8	51.4	48.1	40.5
El Ingenio	27	1885	84.5	36.7	52.1	47.4	39.6
San Vicente	7	1945	81.3	41.4	47.5	50.7	36.5
Mean *P. tecunumanii* (HE)			79.8	42.9	43.3	51.3	33.5
Napite	17	2210	78.6	43.8	42.9	52.6	32.7
San Lorenzo	6	2000	63.0	42.6	37.4	49.4	30.3
Chanal	10	2180	80.5	41.8	46.5	55.9	29.8
Las Piedrecitas	15	2430	74.1	46.3	37.7	56.7	25.9
Cabricán	21	2590	81.0	51.3	33.3	58.6	25.2
La Piedad	24	2155	86.5	52.6	33.7	63.7	21.2
Rancho Nuevo	18	2310	69.8	55.1	24.2	60.0	19.6
El Pinalón	25	2435	73.7	61.4	17.9	67.4	11.7
P. elliottii (susc. FA-2)			115.8	86.3	24.7	106.4	8.6
P. patula			88.6	74.0	14.3	86.2	2.3

[a] Provenances are ordered by 20-week LiveStem. Values for *P. tecunumanii* (HE) provenances are best linear unbiased predictions using 12-week-old seedling height as covariate, values for control species are unadjusted means

thickness and patterns, cone shape, size, and abundance and needle thickness in natural stands (Dvorak 1986; Dvorak and Raymond 1991). Generally, with decreasing altitude of the collection site, needle thickness increases. Camcore has established more than 75 genetic field trials of HE and LE *P. tecunumanii* around the world, and from a perspective of growth and productivity, the results do not conclusively demonstrate that the two *P. tecunumanii* subpopulations should be kept in breeding populations (Hodge and Dvorak 1999). However, it is clear the HE and LE subpopulations react differently to pitch canker infection.

There are important differences in pitch canker resistance at the provenance level for both *P. patula* and HE *P. tecunumanii*, and the variation patterns in both species appear to be related to geography and elevation. For *P. patula*, provenances in the far western and northern part of the range were more resistant than those in the eastern areas (Fig. 1a). For both *P. patula* and HE *P. tecunumanii*, the lower

Fig. 1 (**a**) Relative resistance of *P. patula* provenances to pitch canker. Symbol size represents LiveStem percentage 20 weeks after inoculation of 21-week-old seedlings. Provenance names associated with provenance numbers can be found in Table 1. (**b**) Relative resistance of high-elevation *P. tecunumanii* provenances to pitch canker. Symbol size represents LiveStem percentage 20 weeks after inoculation of 12-week-old seedlings. Provenance names associated with provenance numbers can be found in Table 1b


a
Livestem (%) at 20 weeks
66 - 71
60 - 66
54 - 60
49 - 54
43 - 49
Natural Range
SIERRA MADRE ORIENTAL
SIERRA MADRE DEL SUR
Gulf of Campeche
100 0 100 200 Kilometers
b
Mexico
Belize
Guatemala
Honduras
El Salvador
Pacific Ocean
SAN CRISTOBAL HIGHLANDS
SIERRA MADRE DE CHIAPAS
SIERRA DE CHUACUS
SIERRA DE LAS MINAS
Livestem (%) at 20 weeks
50 - 60
40 - 50
30 - 40
20 - 30
10 - 20
Natural range
50 0 50 100 150 Kilometers

the altitude of the provenance, the more resistant was the genetic material. These results raise an interesting question about what might be the pattern of variation among the LE P. *tecunumanii* provenances—although these provenances are generally very resistant, is there also a relationship between elevation and resistance within this subpopulation?

For *P. patula*, the most resistant provenances occur at the north and southwest tails of the natural distribution (Fig. 1a). The two northernmost populations, El Cielo and Conrado Castillo, are separated from the rest of the *P. patula* natural range by approximately 225 km. Based on our observations in provenance/progeny tests, they differ morphologically from other provenances from the central part of the natural range of *P. patula* in that their crowns are more compact, and their needles are thicker and less pendent. The other two populations in the northern part of the range that show better than average resistance to pitch canker, Pinal de Amoles and La Encarnación, exhibit no discernable external characteristic that would separate them from other *P. patula* populations.

Two southern populations of *P. patula*, Tlacuache and Yextla, exhibit higher levels of resistance than do populations in the central part of the species' natural occurrence, and these provenances are different in several respects. First, they occur in the Sierra Madre del Sur under climatic influences from the Pacific Ocean, rather than under the effects of the more continental climates of Sierra Madre Oriental. Second, their morphology is characterized by smaller cones, shorter needles, more needles per fascicle and a higher proportion of resin canals in the internal position than do populations in the Sierra Madre Oriental (Dvorak et al. 2001). Third, RAPD molecular marker analysis indicates that all of the Yextla and some of Tlacuache trees have unusual marker patterns relative to ''typical'' *P. patula* (Dvorak et al. 2001). However, these ''atypical'' trees are still more closely related to *P. patula* than to any of the other closed-cone pine species. The significance of these marker differences between Yextla and Tlacuache and the other populations in the natural range of *P. patula* populations is not well understood.

For HE *P. tecunumanii*, the populations most susceptible to pitch canker were located in the San Cristobal highlands of Chiapas, Mexico extending into the Sierra de Chuacus and the Sierra de las Minas in north central Guatemala (Fig. 1b). These ranges are predominantly oriented in an east-west direction through both countries (and will be referred to here as the northern range). The most resistant populations of *P. tecunumanii* are located 60–150 km to the south in a second mountain range that runs parallel to the northern range. The southern range is geologically part of the Sierra Madre del Sur that extends from Mexico, across Guatemala and into northern El Salvador (referred here as the southern range). In the western highlands of Guatemala, distinction between the northern and southern ranges is poorly defined, but the two ranges are well separated by the Rio Motagua Valley in southeastern Guatemala.

An attractive hypothesis to explain the trend in resistance among HE *P. tecunumanii* is that there is more *P. oocarpa* in the southern than in the northern range and hybridization and introgression with HE *P. tecunumanii* has produced resistant populations. High levels of introgression might also explain why in DNA bulking assessments by species using RAPD markers, *P. oocarpa* and *P. tecunumanii* show only slight genetic separation (Dvorak et al. 2001), but with no species-specific markers to reliably distinguish between the two groups (Furman and Dvorak 2005). However, the introgression theory is not congruent with the fact that several HE

populations of *P. tecunumanii* in the northern range, like San Lorenzo and Cabrican, occur sympatrically or in close proximity to *P. oocarpa* stands yet exhibit moderate to high susceptibility to pitch canker.

Summary

There is significant and important provenance variation for resistance to pitch canker among *P. patula* and high-elevation *P. tecunumanii*. These differences should be considered when making selection and breeding decisions in these species. In South Africa, some organizations that use *P. patula* as the primary commercial species are considering the possibility of crossing *P. patula* with LE *P. tecunumanii* or *P. oocarpa* to develop pitch canker resistant commercial hybrids. It may be worthwhile to focus on hybrids using material from more resistant provenances.

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