GEOGRAPHIC VARIATION IN STEM-XYLEM TERPENE CHEMISTRY IN NATIVE POPULATIONS OF *PINUS GREGGII* ENGELM.

Jeffrey K. Donahue [1], Jesse P. Perry, Jr.[2], Anthony E. Squillace [3] & Shi Liu [4]

[1] CAMCORE Cooperative, Department of Forestry, North Carolina State University, Box 7626, Raleigh, NC 27695, U.S.A.
[2] Rockefeller Foundation, Retired 306 N. Front St., Hertford, NC 27944, U.S.A.
[3] School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32605 U.S.A.
[4] W3-58 Chemistry West, University of Alberta, Edmonton AB, Canada T6G 2G2

Received October 9, 1995; accepted March 11, 1996

ABSTRACT

Oleoresin samples were collected from 170 individual trees of *Pinus greggii* from 9 native populations located throughout the species' natural distribution in Mexico. The samples were analyzed for chemical composition using gas chromatography and GC mass spectrometry. The species in general had high proportions of ß-phellandrene and low proportions of α-pinene. The northern populations studied were distinct from southern populations in having lower proportions of α-pinene and myrcene, and higher proportions of limonene and longifolene.

Genetic variation in relation to four of the monoterpenes studied was greater in southern populations than in northern populations. Genes affecting the production of the five terpenes studied appeared to be fixed in the northern populations, but only two terpenes tended toward fixation in southern populations.

Key words: *Pinus greggii*, terpenes, geographic variation, *Oocarpae*

INTRODUCTION

Pinus greggii Engelm. has a limited distribution in the mountains of the Sierra Madre Oriental in northeastern and central-eastern Mexico where it occurs at elevations ranging from 1,200 to 2,800 m. SHAW (1909), MARTINEZ (1948) and CRITCHFIELD and LITTLE (1966) reported its occurrence in two distinct regions; the states of Coahuila and Nuevo Leon, and San Luis Potosi and Hidalgo. PERRY (1991) indicated a similar distribution, extending the species range to the states of Veracruz and Puebla, where they border eastern Hidalgo. Other recent works have included previously unreported stands in Queretaro (PLANCARTE 1990; LOPEZ-UPTON and DONAHUE 1995).

Northern and southern populations of *P. greggii* grow in distinct environments. The northern populations grow in colder (≈ 14°C vs. 17 °C average annual temperature), drier climates than the southern region (≈ 650 mm vs. 1,200 mm annual precipitation), and on average at least 600 to 700 meters higher elevation. Topsoil pH at northern sites is usually neutral, or alkaline, while southern sites tend to be more acidic (DONAHUE 1993). Northern populations are separated from the southern populations by almost four degrees latitude.

Provenance/progeny field trials of *Pinus greggii* have been established in Brazil, Colombia and South Africa by the Central America and Mexico Coniferous Resources Cooperative (CAMCORE), North Carolina State University (DVORAK & DONAHUE 1992). After only one year of growth, obvious differences in leaf morphology and color, reproductive phenology and height growth between trees from the northern and southern regions were apparent (DVORAK *et al.* 1996). As a result of these observations, a project was initiated between CAMCORE and the Colegio de Postgraduados, Montecillo, Mexico, with private industry support, to study geographic variation in cone, leaf and seed morphology of *Pinus greggii*, and revise the species' taxonomy if warranted. The analysis of terpene chemistry of conifers is very useful in clarifying taxonomic relations due to the strong genetic control of monoterpene composition, wide variation among and within species, and ease of measurement (HANOVER 1992, SQUILLACE 1981). This paper presents the results of one part of the taxonomic study. The objective was to characterize the terpene chemistry of the species in its native environments.

MATERIALS AND METHODS

Sampling Procedure

Stem-xylem oleoresin was collected in late May and early June of 1993 from 170 trees of *Pinus greggii* from nine widely separated populations, four in central Mexico, and five in northern Mexico. This collection took place at the end of a seasonal five to six month dry period. Collection site locations and descriptions are presented in Figure 1 and Table 1. Samples were collected at 1.3 m stem height by boring a 1 cm diameter hole in the stem, approximately 1 cm deep, and inserting a glass vial into which the oleoresin exuded. The vials were removed approximately 12 hours later, capped using teflon seals, and stored at ambient temperatures until shipment. The samples were from healthy dominant and co-dominant trees in the stands, with a minimum 100 m spacing between them. These same trees were sampled for a study on the cone, leaf and seed morphology of the species (DONAHUE & LOPEZ-UPTON 1996), and seed production (LOPEZ-UPTON & DONAHUE 1995). The samples were transported immediately after collection to North Carolina, USA, and placed in storage at 5 °C until analysis.

Chemical Analysis

Analysis of the resin samples was by gas chromatography (GC), conducted at the Department of Wood and Paper Science, North Carolina State University. The resin samples were dissolved in methylene chloride at a concentration of approximately 1 mg/ml and analyzed on a DB-5 column (J&W Scientific, 30 meters long, ID 0.32 mm, film 0.25 µm), with a Hewlett-Packard 5890 GC with automatic injector, FID detector, and a workstation. The carrier gas was He at a flow rate of 2 $ml \cdot min^{-1}$. Initially the column temperature was held at 45 °C for 1 minute, then increased to 145 °C at the rate of 15 $°C \cdot min^{-1}$. It was then increased at 5 $°C \cdot min^{-1}$. to 260 °C, and held for 10 minutes, and then increased to 270 °C. Meconine was used as the internal standard.

The content of ß-phellandrene and limonene was analyzed on a DB-WAX column (J&W Scientific, 30 meters long, ID 0.32 mm, film 0.25 µm) with the same GC, and under similar chromatographic conditions. Baseline resolution was achieved for these two compounds that could not be separated on the DB-5 column. The chemical components were identified by comparison with known chemical standards, and also compared to results from gas chromatography/ mass

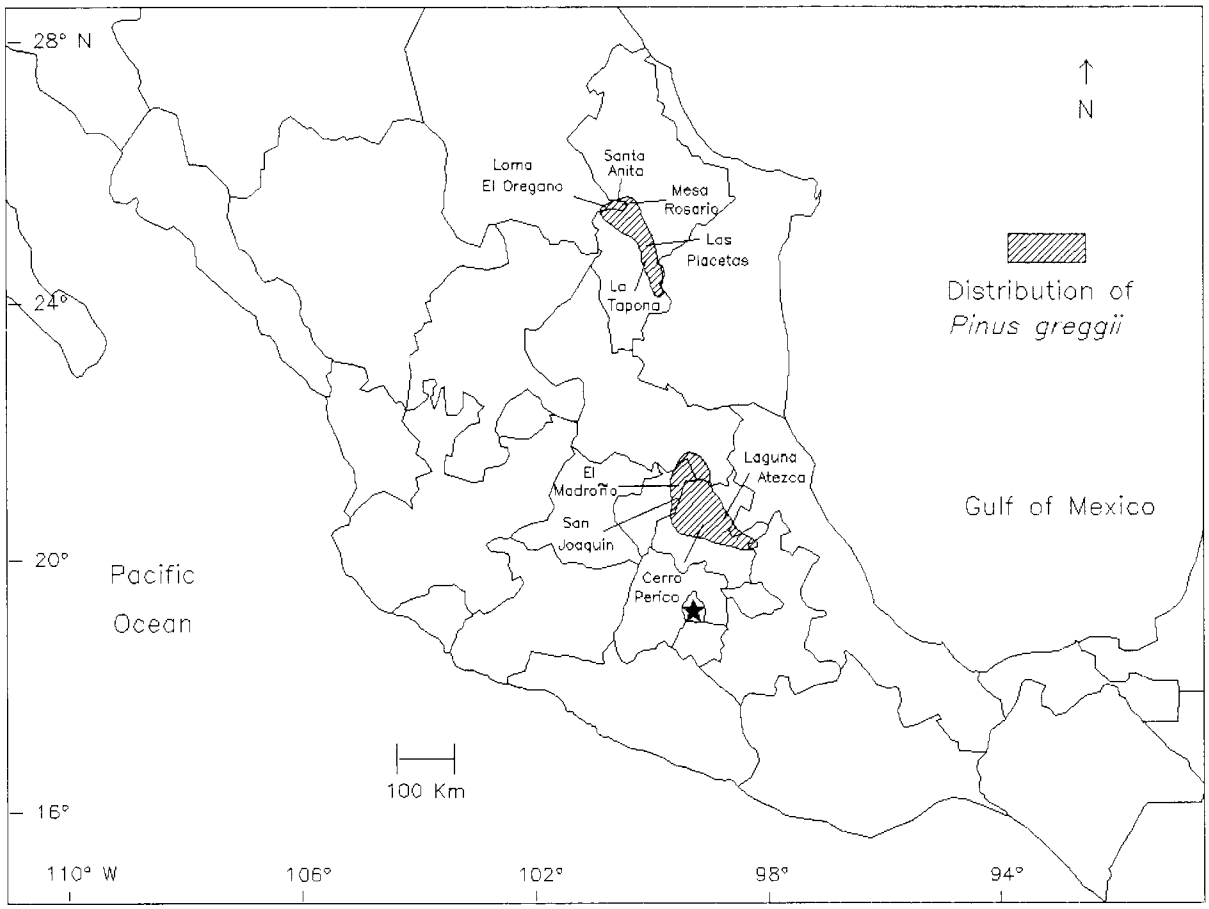

Figure 1 Location of sample sites in Mexico

Table 1 Summary of the *Pinus greggii* sites included in the study

Population	Latitude Longitude	Elevation (m)	Annual precipitation (mm) [1]	Mean annual temperature (°C)	No. of trees sampled
Southern region					
San Joaquin, Queretaro	20°56' N 99°34' W	2,310–2,380	1,100	14	15
El Madroño, Queretaro	21°16' N 99°10' W	1,650–1,730	1,200	17	17
Cerro Perico, Hidalgo	20°44' N 99°02' W	1,830–1,970	800	17	24
Laguna Atezca, Hidalgo	20°49' N 98°46'W	1,250–1,420	1,650	19	16
Northern region					
Santa Anita, Coahuila	25°27' N 100°34'W	2,515–2,620	650	13	20
Mesa del Rosario, Coahuila	25°26' N 100°28'W	1,920–2,325	650	13	13
Loma El Oregano, Coahuila	25°22' N 100°55'W	2,310–2,350	600	13	19
Las Placetas, Nuevo Leon	24°55' N 100°11'W	2,370–2,520	750	16	22
La Tapona, Nueva Leon	24°43' N 100°10'W	2,090–2,350	650	15	24

[1] Data from INEGI (1980)
[2] Includes dispersed stands which occur from Agua Fria (25° 24' N, 100° 25'W) to El Tarrillal (25° 27' N, 100° 31'W)

spectrometry analyses run on one sample from each of nine populations in the study [1]. The chemical compositions are reported as "percent of terpenes". This includes all the monoterpenes and longifolene, a sesquiterpene, which was of particular interest in this study. This procedure did not separate myrcene and carene, a result which requires relatively high column temperatures. We report a combined amount for both, denoted as myrcene/carene in the tables, since they elute at almost the same time (SQUILLACE 1976), and we believe there is a small amount of carene in some trees.

RESULTS AND DISCUSSION

Terpene Composition

Four of the monoterpenes and one sesquiterpene were found in significant quantities and/or varied appreciably by geographic location: α-pinene, myrcene, limonene, ß-phellandrene and longifolene (Table 2). ß-phellandrene was the single most abundant terpene in all populations (average 52% relative content). Although there was much more variation among and within southern populations of *P. greggii* than northern ones, on average they had higher amounts of α-pinene (15% vs. 5%) and myrcene (13% vs. 2%) than northern populations, and lower amounts of limonene (9% vs. 32%) and longifolene (0.2% vs. 4.3%). ß-pinene was found in low proportions in all populations.

Other monoterpenes which were detected in small or trace amounts included tricyclene, camphene, sabinene, α-phellandrene, terpinolene, linalool, α-turpinol and caryophyllene. Small amounts of γ-cadinene and methyl-chavicol were detected in southern populations, but not in northern populations.

These results agree well with the only other terpene study done on *Pinus greggii* (LOCKHART 1990). She found similar patterns of high ß-phellandrene and limonene and low α-pinene in the northern population Las Placetas, Nuevo Leon, which was common to both studies. Lockhart's work, based on 33 trees from the one population, gave average contents of α-pinene, ß-pinene, carene, limonene, ß-phellandrene, and longifolene of 7, 1, 1, 32, 45 and 6% respectively.

In order to characterize individual trees as "high" or "low" types for specific terpenes, frequency distributions were examined to help establish threshold values as criteria for determining the point of separation for the

[1] GCMS analyses conducted by SCM GLIDCO Organics Corporation, Jacksonville, FL., USA.

Table 2 Population means and (*ranges*) for percent terpene content of *Pinus greggii* [1]

Population	α-pinene	β-pinene	myrcene/ carene	limonene	β-phellandrene	longifolene
Southern region:						
San Joaquin	36.5 *(4.5–67.0)*	0.7 *(0.0–1.0)*	23.5 *(1.8–46.1)*	2.1 *(0.6–17.3)*	23.6 *(0.7–80.7)*	0.11 *(0.00–0.30)*
El Madroño	8.3 *(4.0–36.7)*	0.7 *(0.3–1.1)*	7.8 *(2.0–26.2)*	16.6 *(1.0–42.3)*	59.4 *(25.1–78.5)*	0.25 *(0.16–0.38)*
Cerro Perico	8.3 *(4.4–38.7)*	0.9 *(0.6–1.2)*	18.0 *(1.3–35.0)*	4.3 *(1.1–27.4)*	61.2 *(34.7–81.7)*	0.24 *(0.15–0.32)*
Laguna Atezca	5.4 *(4.4–7.1)*	0.7 *(0.6–0.9)*	4.1 *(1.6–21.3)*	12.2 *(1.7–24.7)*	71.8 *(52.5–84.4)*	0.22 *(0.18–0.30)*
Mean:	**14.6**	**0.75**	**13.3**	**8.8**	**54.0**	**0.20**
Northern region:						
Santa Anita	4.3 *(3.3–5.8)*	0.7 *(0.6–1.0)*	2.2 *(2.1–2.7)*	31.3 *(1.5–43.5)*	50.6 *(37.1–79.1)*	4.81 *(3.14–7.09)*
Mesa El Rosario	5.0 *(3.5–7.3)*	0.7 *(0.5–0.8)*	2.1 *(2.0–2.4)*	28.9 *(13.4–42.0)*	52.9 *(42.0–60.1)*	4.33 *(2.03–6.42)*
Loma El Oregano	5.0 *(3.5–10.0)*	0.5 *(0.4–0.7)*	2.2 *(2.1–2.5)*	32.6 *(22.6–42.0)*	49.5 *(41.3–59.8)*	5.57 *(3.66–7.43)*
Las Placetas	5.5 *(4.6–7.1)*	0.6 *(0.5–0.9)*	2.1 *(1.9–2.3)*	26.5 *(12.0–39.7)*	55.8 *(42.3–68.5)*	2.60 *(1.09–5.03)*
La Tapona	4.2 *(3.5–5.5)*	0.5 *(0.4–0.7)*	2.4 *(2.1–2.6)*	36.2 *(22.2–43.5)*	47.6 *(40.6–59.8)*	4.45 *(2.36–10.38)*
Mean:	**4.7**	**0.61**	**2.2**	**31.6**	**50.9**	**4.35**

1 Relative to the total sum of monoterpenes plus longifolene

concentration classes (per SQUILLACE 1976) (Figures 2a–2e). As seen in the figures, frequency distributions for all five terpenes show indications of bimodality, though evidence is not as clear in ß-phellandrene (Figure 2c). Threshold values were established at points where the trees' frequency distributions might be separated into two modes.

The wide variation seen in the frequency distributions is due mainly to differences between southern and northern groups of populations. For example, all of the northern trees had low α-pinene and myrcene, while both high and low types were found only in southern populations (Figures 2a & 2b). For α-pinene, 80% of the high-concentration trees were from the San Joaquin population. The small group of low ß-phellandrene trees seen in Figure 2c were all from the southern population San Joaquin (Table 3). All but one of the northern trees had high limonene, while both high and low types occurred in the south (Figure 2d, Table 3). For longifolene, the distinction is complete; northern populations had all high-concentration trees and southern populations had all low-concentration trees (Figure 2e, Table 3).

Most notable in Table 3 is that the northern populations had practically no variation in frequency of high and low concentration types; only one population (Santa Anita) had both high and low types, and only for limonene. Thus it appears that the gene or genes controlling these terpenes are fixed in the northern populations of *Pinus greggii*. Southern populations differ from northern populations by having variable numbers of trees with high and low concentrations of α-pinene, myrcene and limonene, and in one population, ß-phellandrene.

RAMÍREZ-HERRERA (1993) obtained similar results in a study of genetic variation in isoenzymes of *P. greggii*. Based on genetic distances, northern populations separated into a distinct group from southern populations. Over 50% of the loci he studied showed strong tendencies toward fixation, which, on average, is a higher percentage than in most pines. In his study both extreme values in numbers of polymorphic loci were found in southern populations.

The higher amount of variation in the southern populations of *P. greggii* could be a result of introgression with *P. patula* Schiede & Deppe. Their ranges overlap in central Mexico, and they are the two most closely related Mexican species in the closed-cone pine group. Successful artificial crosses between the two species have been reported (CRITCHFIELD 1966, FIELDING 1960). Although both species occur in northeastern

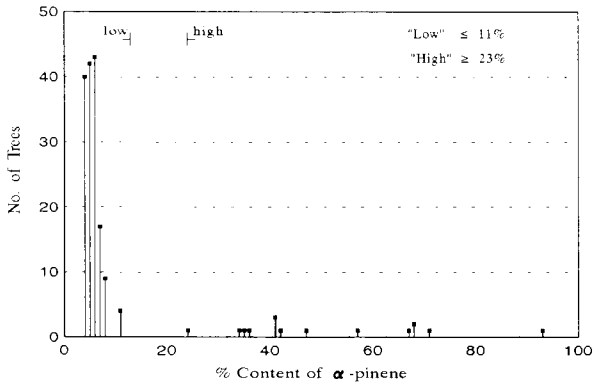

Figure 2a. Frequency distribution for α-pinene.

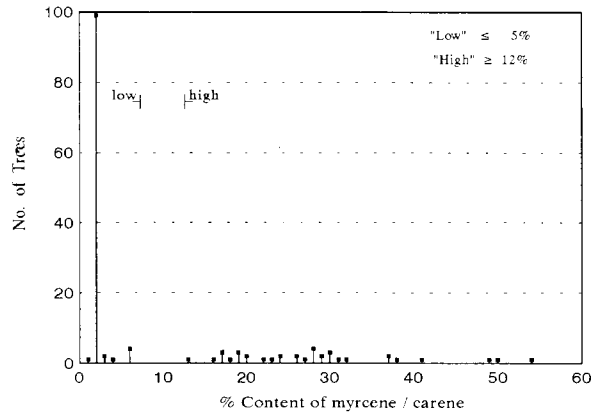

Figure 2b. Frequency distribution for myrcene & carene.

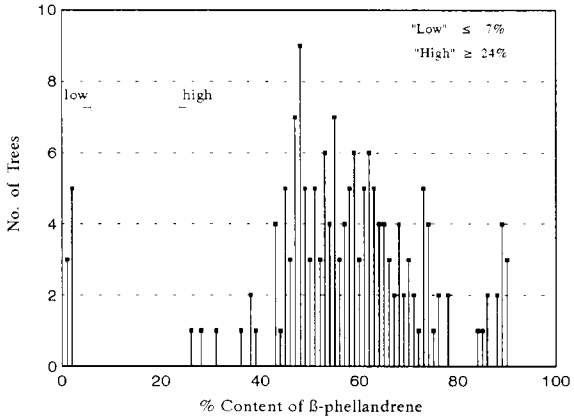

Figure 2c. Frequency distribution for β-phellandrene.

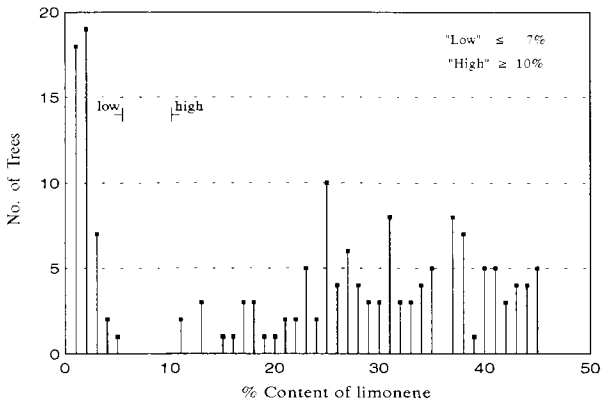

Figure 2d. Frequency distribution for limonene.

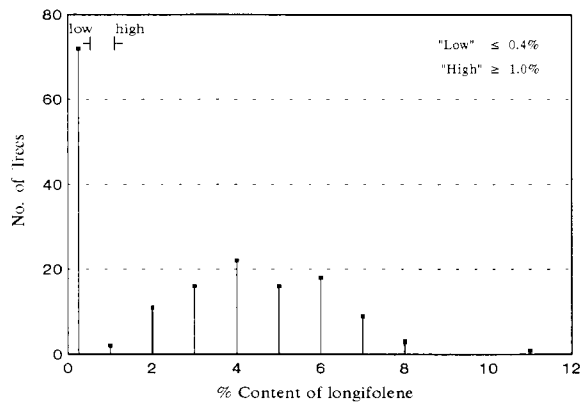

Figure 2e. Frequency distribution for longifolene.

Mexico, they have not been reported growing together.

Ninety-five percent of all trees had high ß-phellandrene, and 91% had low α-pinene, results which characterize the species in general as high ß-phellandrene and low α-pinene. However, there are other differences which distinguish southern populations from northern populations. Fifty percent of trees in southern populations had high myrcene, while northern populations had no high-myrcene trees. In southern populations 36% of the trees had high limonene, while in northern populations 99% of the trees had high amounts of limonene.

The results in Table 3 show that northern populations of *P. greggii* have high limonene and high longifolene, while southern populations have predominately low limonene and low longifolene. The one terpene that clearly distinguishes northern populations from southern ones is longifolene. All northern-region trees were classified as having high concentrations,

Table 3 Number of trees with high ant low amounts of each terpene by population

Population	high – low α-pinene	high – low myrcene/carene	high – low limonene	high – low β-phellandrene	high – low longifolene
Southern region:					
San Joaquin	12 – 3	12 – 3	1 – 14	7 – 8	0 – 15
El Madroño	1 – 16	4 – 13	13 – 4	17 – 0	0 – 17
Cerro Perico	2 – 22	18 – 6	3 – 21	24 – 0	0 – 24
Laguna Atezca	0 – 16	2 – 14	9 – 7	16 – 0	0 – 16
Total number of trees:	15 – 57	36 – 36	26 – 46	64 – 8	0 – 72
Northern region:					
Santa Anita	0 – 20	0 – 20	19 – 1	20 – 0	20 – 0
Mesa El Rosario	0 – 19	0 – 19	19 – 0	19 – 0	19 – 0
Loma El Oregano	0 – 22	0 – 22	22 – 0	22 – 0	22 – 0
Las Placetas	0 – 24	0 – 24	24 – 0	24 – 0	24 – 0
La Tapona	0 – 13	0 – 13	13 – 0	13 – 0	13 – 0
Total number of trees:	0 – 98	0 – 98	97 – 1	98 – 0	98 – 0

while all southern trees had low concentrations. This difference could be used to determine the provenance region of unidentified seedlots. "Relative" amounts of terpenes in pines have been shown to be under strong genetic control (TOWNSEND & HANOVER 1972, SQUILLACE 1976). Knowledge of seed origin in *P. greggii* is important to tree planters since trees from northern populations grow significantly slower than southern trees in some subtropical environments (DVORAK *et al.* 1996, DVORAK & DONAHUE 1992).

The apparent fixation of alleles for low longifolene in southern populations and for high longifolene in northern populations is especially interesting. It is possible that some environmental selection pressure has acted on the species as it migrated between the dry, cool environments of northeast Mexico, and the warmer, more humid sites in east-central Mexico. Northern stands of *P. greggii* occur in relative isolation from other related pine species, while southern populations are found in closer proximity to other pines such as *P. patula* and *P. teocote* Schiede & Deppe. Monogenic control of production of longifolene has been demonstrated for *Pinus pinaster* Ait. (MARPEAU *et al.* 1975). If this is the case with *P. greggii*, this could expedite the process of fixation in many of the species populations due to their small size and isolation. The appearance of bimodality in the other terpenes studied also indicates monogenic control over their production. Evidence has been presented supporting this mode of inheritance for other *Pinaceae* species (SQUILLACE 1981).

Of all populations studied, San Joaquin was the most variable. It diverged from the general trend across all populations for concentrations of α-pinene, myrcene, limonene and ß-phellandrene. This population grows at the highest elevation reported to date for the species in central Mexico (2,400 m). It is the western outlier in the southern region, and is located about 35 km west of the nearest population (El Piñon, Hidalgo) effectively isolated from any other *P. greggii* population. Although there is no evidence of introgression from other species to explain the increased variation at San Joaquin, there are large stands of *P. teocote* located 1 km south, along with scattered individuals of *P. montezumae*.

These results coincide with a study on cone, leaf and seed morphology which was conducted on material from the same trees from which the oleoresin was collected. Results from discriminant analyses using 17 traits showed that the southern populations were morphologically different from the northern populations, and were more variable (DONAHUE & LOPEZ-UPTON 1996). In multivariate analyses of variation, needle length, needle width, number of stomata, number of internal resin canals, seed wing width, seed weight and seed coat thickness were found to be useful in discriminating northern trees from southern trees.

Statistics

Four-fold chi-square analyses (SNEDECOR & COCHRAN 1989) were done on the ratios of high and low trees to check for differences between populations in which similarities were apparent. Among southern populations, there was no difference between San Joaquin and Cerro Perico in ratios of high/low trees in myrcene (χ^2 = 0.564) and limonene (χ^2 = 1.269). El Madroño and Laguna Atezca also had similar ratios for myrcene (χ^2 = 0.153) and limonene (χ^2 = 0.743). Laguna Atezca and

Table 4 A comparison of terpene composition of latin American closed cone pines. All values have been standardized to reflect percent terpene content relative to the total of those terpenese included in the table

Species / (Locations)	α-pinene	β-pinene	myrcene/ carene	limonene	β-phellandrne	longifolene
Pinus greggii [1] (southern populations)	17	0.8	15	10	58	0.3
Pinus greggii [1] (northern populations)	5	0.7	3	41	54	5
Pinus patula [2] (Mexico)	8	1	2	2	77	9
Pinus oocarpa [2] (Mexico, Guatemala; Honduras; Nicaragua)	78	7	3	1	2	10
Pinus tecunumanii [2] (Mexico; Honduras; Guatemala)	22	1	11	29	26	11
Pinus pringlei [3] (Oaxaca, Mexico)	45	15	–	0.6	3	10

[1] Results from this study; [2] Results from Squillace and Perry (1992); [3] Results from Lockhart (1990)

Cerro Perico did have significantly different ratios for limonene ($\chi^2 = 10.96$; $\alpha = 0.025$).

Attempts to effect a normal distribution in the data through arcsine-square root, natural logarithm, and log-ratio (AITCHISON 1984) transformations were unsuccessful. Thus no inferences using multivariate analyses were attempted on the data for these terpenes. Further consideration will be given to analyzing these results using methods based on maximum likelihood principals such as likelihood ratio tests (CASELLA & BERGER 1990). This procedure could prove useful in analyzing differences among populations using the ratios of trees with high and low concentrations.

Relationships with other species

Table 4 gives a comparison of terpene chemistry for the predominant closed-cone pine species in Mexico and Central America. In terms of relative amounts, *Pinus greggii* is most similar to *P. patula* in α-pinene and ß-phellandrene content. It has comparable amounts of ß-pinene to *P. patula* and *P. tecunumanii* (Schw.) Eguiluz & Perry. However, in relative amounts of limonene southern populations of *P. greggii* resemble *P. patula*, while northern populations are more similar to *P. tecunumanii*. Since the range of *P. patula* overlaps that of *P. greggii*, natural crossings between the species may account in part, for the high levels of ß-phellandrene found in both species. However, apart from limonene, the northern *P. greggii* populations are more similar to *P. patula* than the southern populations, despite the present day scarcity of *P. patula* in the north. The terpene chemistry of *P. greggii* is distinct from *P. oocarpa* Schiede and *P. pringlei* Shaw in α-pinene, ß-pinene and ß-phellandrene, but similar in longifolene.

Since the first studies, taxonomists have noted the similarities between *Pinus greggii* and *P. patula* in their cones and other characteristics (SHAW 1914, MARTINEZ 1948, MIROV 1967). LITTLE and CRITCHFIELD (1969) grouped *P. greggii*, *P. patula*, *P. pringlei*, *P. oocarpa*, *P. radiata* D. Don, *P. attenuata* Lemm. and *P. muricata* D. Don together in *Pinus* subsection *Oocarpae*. Morphologically, *P. greggii* seems to fit well in the closed-cone group of Mexican pines, however the only unifying chemical pattern for these species appears to be that of relative amounts of ß-pinene and longifolene.

Although the cones of *P. greggii* are very similar to those of *P. patula*, *P. greggii* and *P. attenuata* are even more alike morphologically than *P. greggii* and *P. patula* in cones, needles and the smooth upper stem bark. Chemical patterns, however, are completely different for the two species; *P. attenuata* has very high α-pinene levels and low levels of other terpene components (MIROV 1961, FORDE 1964), compared to low levels of α-pinene, and high ß-phellandrene and limonene in *P. greggii*.

In a phylogenetic analysis of closed-cone pines using random amplified polymorphic DNA markers, GRATTAPAGLIA *et al.* (1993) found very little amount of DNA sequence polymorphism between southern and northern populations of *P. greggii*. However the species was highly polymorphic in relation to *P. oocarpa*, *P. tecunumanii*, *P. pringlei*, and *P. patula*.

CONCLUSIONS

In terms of terpene chemistry, the southern populations of *Pinus greggii* included in this study are different from northern populations in relative amounts of α-pinene, myrcene, limonene and longifolene. Genetic variation related to production of some terpenes appears

to be much lower in the northern populations than in the southern populations. Genes affecting relative amounts of ß-phellandrene and longifolene appear to be fixed, or nearly fixed in almost all populations. The genes affecting amounts of five principal chemical components appear to be fixed in the northern populations. This study indicates that potential exists for distinguishing trees from northern and southern populations based on their terpene chemistry.

If in fact the northern populations have less genetic variation, the cause remains to be determined. It is possible that these populations are presently being reduced and isolated by long-term environmental changes, causing fixation of certain gene complexes. Or it is possible that present day populations are descendants of previously reduced populations and the sites have been repopulated with relatively few genotypes. Recent events indicate that northern populations are more subject to extreme changes in the environment than southern populations as evidenced by the present drought conditions in northern Mexico persisting at least since 1993. Many *P. greggii* stands now have significant pest and disease problems, possibly caused by drought stress. If the level of genetic variation is as different among populations as indicated by these studies, gene conservation strategies for this important species will have to take this into account to be most effective. It could be that fewer trees would be required from northern populations than from southern populations to conserve a majority of their genetic variation.

ACKNOWLEDGEMENTS

The following institutions and organizations made the financial or logistical contributions which made this study possible: College of Forest Resources, North Carolina State University, USA; Colegio de Postgraduados, Mexico; Mondi Forests, South Africa; SCM Glidco Organics Corporation, USA; and the *Central America and Mexico Coniferous Resources Cooperative* (CAMCORE), North Carolina State University, USA. Jesse Perry also provided financial assistance.

The authors wish to express their appreciation to Javier Lopez-Upton (Colegio de Postgraduados) for his assistance in the field sampling, and to Chen Loung Chen (NCSU) and C. Rodney Gorman (SCM Glidco Organics Corp.) for their assistance with the chemical analyses and interpretation. We also extend our thanks to William Dvorak and Jorge Vasquez (CAMCORE) for their assistance with data interpretation and review of the manuscript.

REFERENCES

AITCHISON, J. 1984: The statistical analysis of geochemical compositions. *Mathematical Geology* **16**:531–564.

CASELLA, G. & BERGER, R. L. 1990: Statistical inference. Wadsworth and Brooks/Cole, Pacific Grove, CA. 650 pp.

CRITCHFIELD, W. B. 1966: Crossability and relationships of the closed-cone pines. *Silvae Genetica* **16**:89–97.

CRITCHFIELD, W. B. & LITTLE, E. L. 1966: Geographic Distribution of Pines of the World. USDA For. Serv. Misc. Publ. 991.

DONAHUE, J. K. 1993: Geographic variation in *Pinus greggii* seedlings in relation to soil acidity. *In:* Proceedings IUFRO Conference Breeding Tropical Trees: Resolving Tropical Forest Resource Concerns Through Tree Improvement, Gene Conservation and Domestication of New Species. Cartagena and Cali, Colombia, October, 1993. pp 172–177.

DONAHUE, J. K. & LOPEZ-UPTON, J. 1996: Geographic variation in leaf, cone and seed morphology of *Pinus greggii* in native forests. *Forest Ecol. Manage.* (in press)

DVORAK, W. S. & DONAHUE, J. K. 1992: CAMCORE Cooperative Research Review, 1980–1992. Col. of For. Resources, North Carolina State University, Raleigh. 93 pp.

DVORAK, W. S., KIETZKA, E. & DONAHUE, J .K. 1996: Three-year survival and growth of provenances of *Pinus greggii* in the tropics and subtropics. *Forest Ecol. Manage.* (in press)

FIELDING, J. M. 1960: *Pinus patula* x *greggii*. *Austral. Forest.* **24**:99–102.

FORDE, M. B. 1964: Inheritance of Turpentine Composition in *Pinus attenuata* x *radiata* hybrids. *New Zealand Jour. of Bot.* **2**(1):53–59.

GRATTAPAGLIA, D., O'MALLEY, D. & DVORAK, W. 1993: Phylogenetic analysis of Central American and Mexican pines using RAPD markers on bulked DNA samples. *In:* Proceedings IUFRO Conference Breeding Tropical Trees: Resolving Tropical Forest Resource Concerns Through Tree Improvement, Gene Conservation and Domestication of New Species. Cartagena and Cali, Colombia, October, 1993. pp 132–147.

HANOVER, J. W. 1992: Applications of terpene analysis in forest genetics. *New Forests* **6**:159–178.

INEGI 1980: Atlas Nacional del Medio Físico. Carta de Climas. Primera Impresión. INEGI, México, D.F.

LITTLE, E. L. & CRITCHFIELD, W. B. 1969: Subdivisions of the Genus *Pinus*. USDA For. Serv. Misc. Publ. No. 1144. 51 pp.

LOCKHART, L. A. 1990: The xylem resin terpene composition of *Pinus greggii* Engelm. and *Pinus pringlei* Shaw. *Silvae Genetica* **39**:198–202.

LOPEZ-UPTON, J. & DONAHUE, J. K. 1995: Seed production of *Pinus greggii* in natural stands in Mexico. *Tree Planter's Notes, USA* **46**(3): (in press).

MARPEAU, A., BARADAT, PH. & BERNARD-DAGAN, C. 1975: Les terpènes du pin maritime: aspects biologiques et génétiques. IV. Hérédité de la teneur en deux sesquiterpènes: le longifolène et le caryophyllène. *Ann. Sci. For.*, **32**(4):185–203.

MARTÍNEZ, M. 1948: Los Pinos Mexicanos. Segunda Edición, Ediciones Botas. México, D.F.

MIROV, N. T. 1961: Composition of Gum Terpentines of Pines. USDA For. Serv. Tech. Bull. 1239. 158 pp.

MIROV, N. T. 1967: The Genus *Pinus*. The Ronald Press Co. New York. 602 pp.

PERRY, J. P. 1991: The Pines of Mexico and Central America. Timber Press. Inc. 231 pp.

PLANCARTE, A. 1990: Variación en longitud de cono y peso de semilla en *Pinus greggii* Engelm. de tres procedencias de Hidalgo y Querétaro. Nota Técnica No. 4. Centro de Genética Forestal, A. C., Chapingo, México. 6 pp.

RAMÍREZ-HERRERA, C. 1993: Evaluación de la diversidad genetica en poblaciones naturales de *Pinus greggii*. MS Thesis. Chapingo. México. 90 pp.

SHAW, G. R. 1909: The Pines of Mexico. *Journal of the Arnold Arboretum* **1**. 30 pp.

SHAW, G. R. 1914: The Genus *Pinus*. *Journal of the Arnold Arboretum* **5**. 96 pp.

SNEDECOR, G. W. & COCHRAN, W. G. 1989: Statistical Methods. Iowa State University Press. Ames. 503 pp.

SQUILLACE, A. 1976: Analyses of monoterpenes of conifers by gas-liquid chromatography. *In:* J. P. Miksche (ed.). Modern Methods in Forest Genetics. 288 pp. New York. Springer Verlag.

SQUILLACE, A. 1981: Inheritance of monoterpene composition in conifers. In: Advances in Forest Genetics. Ambika. New Dehli, India. 375 pp.

SQUILLACE, A. E. & PERRY, J. P. 1992: Classification of *Pinus patula*, *P. tecunumanii*, *P.oocarpa*, *P. caribaea* var. *hondurensis*, and Related Taxonomic Entities. USDA For. Serv., Southeastern For. Experiment Sta. Research Paper SE–285, 23 pp.

TOWNSEND, A. M. & HANOVER, J. W. 1972: Altitudinal variation in photosynthesis, growth, and monoterpene composition of western white pine (*Pinus monticola* Dougl.) seedlings. *Silvae Genetica* **21**(3–4):133–139.