eastern Forest Exp. Stn., Asheville, NC. 26p. (1980). —— Smouse, P. E. and Saylor, L C.: Studies of the *Pinus rigida-serotina* complex. II. Natural hybridization among the *Pinus rigida-serotina* complex, *P. taeda* and *P. echinata*. Ann. Missouri Bot. Garden **60** (2): 192—203 (1973). —— Snow, G. A. and Froelich, R. C.: Daily and seasonal dispersal of basidiospores of *Cronartium fusiforme*. Phytopatology **58**: 1532—1536 (1968). —— Snow, G. A., Wells, O. O. and Switzer, G. L.: Fusiform rust gradient in a loblolly pine plantation. For. Sci. **32**: 372—376 (1986). —— Snyder, E. B., Wakely, P. C. and Wells, O. O.: Slash pine provenances tests. J. For. **65**: 414—420 (1967). —— Squillace, A. E.: Geographic patterns of fusiform rust infection in loblolly and slash pine plantations. USDA Forest Serv. Res. Note SE-232. Southeastern Forest Exp. Stn., Asheville, NC. 4p. (1976). —— Squillace, A. E. and Kraus, J. F.: Early results of a seed source study of slash pine in Georgia and Florida. Proc. 5th Southern Forest Tree Improvement Conf. 1959: 21—34 (1959). —— Strauss, S. H., Neale, D. B. and Wagner, D. B.: Genetics of the chloroplast in conifers. J. Forestry **87**: 11—17 (1989). —— Symposium on Management of Fusiform Rust: Influence of climate on fusiform rust incidence and distribution. Proc.. Symp. on Management of Fusiform Rust in Southern Pines. (Gainesville, FL., Dec. 1978). University of Florida, Gainesville, FL p. 80 (1977). —— Tukey, J. W.: Quick and dirty methods in statistics. Part II. Simple analyses for standard designs. Proc. 5th Ann. Convention, Amer. Soc. for Quality Control. pp. 189—197 (1951). —— Watts, W. A.: Vegetational history of the eastern United States 25,000 to 10,000 years ago. *In*: S. C. Porter (ed.). The Late Pleistocene. p. 294—310. Vol. I. Late-Quaternary environments of the United States. University of Minneapolis Press, Minneapolis (1983). —— Wells, O. O., Nance, W. L. and Thielges, B. A.: Variation in needle traits in provenance tests of *Pinus taeda* and *P. echinata*. Silvae Genetica **26**: 125—130 (1977). —— Wells, O. O. and Wakeley, P. C.: Geographic variation in survival, growth and fusiform-rust infection of planted loblolly pine. For Sci. Monogr. **11**, 40 p. (1966). —— Wilcox, J. R.: Sweetgum seed stratification requirements related to winter climate at seed source. For. Sci. **14** (1): 16—19 (1968). —— Wright, J. W.: Introduction to Forest Genetics. Academic Press, Inc., New York. 463 p. (1976). —— Yates, F.: The analysis of replcated experiments when the field results are incomplete. Am. J. Exp. Agric. **1**: 129—142 (1933).

A Simple and Rapid Method for Estimating Representation of Species in Spruce Seedlots using Chloroplast DNA Restriction Fragment Length Polymorphism

By B. C. S. Sutton[1]), D. J. Flanagan[1]) and Y. A. El-Kassaby[2])

(Received 18th June 1990)

Abstract

Spruce seedlots containing species mixes and hybrids of Sitka spruce (*Picea sitchensis* [Bong.] Can.) and interior spruce (*Picea glauca* [Moench] Voss/*P. engelmanni* [Parry]) produce seedlings of unacceptable stocktype under operational nursery growing regimes in British Columbia. We have investigated the utility of chloroplast DNA (cpDNA) restriction fragment length polymorphisms for identification of the species composition of these seedlots. A *BamHI* library of Sitka spruce cpDNA was constructed in pUC8. Two clones were selected by hybridization with a 10.5kb *BamHI* fragment of white spruce cpDNA which is unique to interior spruce. One of these (pSS4) containing a 4.3kb *BamHI* fragment was tested in screening of pure and mixed seedlots of Sitka and interior spruce. The results show that this probe can be used to screen total DNA samples to reliably identify and quantify the cpDNA composition of two week old germinants using a sample size of 0.5 g and allows less than 5% species contamination to be detected. Analysis of seedlings from a hybrid seedlot showed that both chloroplast types could be found in some individuals. This result demonstrates the occurrence of hybrid individuals in seedlots and suggests that chloroplasts can be biparentally inherited in *Picea* ssp. Seedlot identification obtained with the cpDNA probe agreed with the recommended growing regimes based on the nursery performance of the seedlings.

Key words: Picea, chloroplast, DNA, polymorphism, hybrids.

[1]) Forest Biotechnology Centre, British Columbia Research Corporation, 3650 Wesbrook Mall, Vancouver, B. C., V6S 2L2, Canada.

[2]) Saanich Forestry Centre, Canadian Pacific Forest Products, Ltd., 8067 East Saanich Rd., RR#1, Saanichton, B. C., V0S 1M0, Canada.

Introduction

Approximately 100 million spruce seedlings are produced for reforestation operations in British Columbia annually. Seed collections from natural stands of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and interior spruce (comprising the white/Engelmann complex; *Picea glauca* [Moench] Voss/P. *engelmannii* [Parry] provide the majority of seed sown in British Columbia nurseries. The overlap of natural ranges of Sitka spruce and interior spruce and the lack of reproductive barriers to hybridization have permitted the creation of several introgression zones (Daubenmire, 1968; Roche, 1969, Krajina et al., 1982). About 6 million spruce seedlings are produced annually from seedlots collected from the coastal zone of introgression between Sitka and interior spruce. Since these seedlots are sown under the same nursery growing regime, the production of seedlings of unacceptable quality is expected due to the different cultural requirements of each species (Brix, 1972). A. reliable and cost effective means of screening these seedlots would allow mixed or hybrid seed to be grown under suitable conditions for the predominant species or to be discarded, and result in saving most of the loss now experienced. Several studies have been pursued to develop a reliable screening method which would allow accurate classification of spruce seedlots (see El-Kassaby, 1988, for review).

Recently it has been shown that chloroplast (cp)DNA restriction fragment analysis can provide a tool for determining the presence of hybrid progeny (Szmidt et al., 1987; Wagner et al., 1987; Szmidt et al., 1988; El-Kassaby et al., 1988). Analysis of inheritance in some gymnosperms have revealed that, in contrast to angiosperms, cpDNA is paternally inherited (Neale et al., 1986; Neale et al., 1989; Neale and Sederoff, 1989; Szmidt et al., 1987; Wagner et al., 1989).

We have isolated a cloned cpDNA fragment from Sitka

spruce that can be used in a routine, cost-effective method for analyzing hybrid and/or mixed spruce seedlots prior to nursery sowing. Results of screening individuals from a hybrid seedlot suggest that biparental inheritance of chloroplasts can occur frequently in spruce.

Materials and Methods

Plant material

Samples of mature individuals of white spruce and ENGELMANN spruce were obtained from the British Columbia Ministry of Forests research station at Vernon, B.C. In view of the hybridization which commonly occurs between white and ENGELMANN spruce in British Columbia, the following criteria were used for species classification of these individuals: The white spruce is a clone derived from scion collected in Ontario, 44°50'N 83°45'W elevation 245 m, where hybridization with ENGELMANN spruce does not occur. The ENGELMANN spruce was collected from the East Kootenay region, 49°05'N 115°29'W, elevation 1,435 m, and has cone morphology typical of ENGELMANN spruce. Sitka spruce was obtained from a wild stand at Sooke, Vancouver Island, B.C. (48°21'N, 123°50'W, elevation 20 m). These samples were used as controls for species type in all experiments. Seedlots and seedlings grown from seedlots were obtained from the BC Ministry of Forests Seed Centre and nursery at Surrey, B.C. The seedlots are seed collections from wild stands (with the exception of one from a seed orchard) the locations of which are presented in *table 1* and *figure 5*.

The seed was sterilized and stratified at 4 °C for 3 weeks. Germination was then allowed to proceed on damp paper towels for 2 weeks at which time the shoots were harvested for total DNA extraction.

DNA extraction

Chloroplast DNA was extracted following the protocol of WHITE (1988). Briefly, 100 g of needles were ground in liquid nitrogen and suspended in extraction buffer. Following centrifugation the pellet containing chloroplasts was resuspended and the chloroplasts purified by sucrose density gradient centrifugation. Pelleted material was also recovered and used as a source of nuclear DNA. Total DNA was prepared by the method of WAGNER et al. (1987).

Recombinant DNA methods

Unique *BamHI* cpDNA restriction fragments of Sitka and white spruce were identified by electrophoresis on 0.8 % agarose gels containing TAE buffer (MANIATIS et al., 1982). The 10.5 kb cpDNA fragment from white spruce cpDNA and inserts of clones pSS4 and pSS6 were isolated for use as hybridization probes. Restriction fragments were isolated from 1% low melting point agarose gels and labelled by the oligolabelling method of FEINBERG and VOGELSTEIN (1983, 1984) to a specific activity of $2\text{-}5\times10^9$ dpm/μg. A library of Sitka spruce cpDNA was prepared

Table 1. — Summary of analysis of spruce seedlots using cpDNA probe pSS4 on total genomic DNA extracted from 200, 2-week-old germinants or 100, 1-year-old seedlings.

	Seedlot Registration			Identification			
				Present Study		Woods (1988)	
Material	#	Species[1]	Location	Species	% interior spruce	Species	Recommended growing regime
Seed	1855	Sxs	Skeena Crossing	Sx	40	Sx	Ss
	1873	Sxs	Shames River	Ss	0	Sx	Ss
	2647	Ss	Noeick River	Sx	<5	Ss	Ss
	2653	Sxs	Shulbuckha	Ss	0	Ss	Ss
	2762	Sxs	Carnaby	Sx	50	Sx	Ss
	2793	Sxs	Kitimat Valley	Ss	0	Ss	Ss
	2856	Sxs	Lakelse River	Ss	0	Ss	Ss
	3307	Sxs	TFL 17	Ss	0	Ss	Ss
	3969	Sxs	Hankin Creek	Sx	<5	Sx	Ss
	3997	Ss	Bella Coola	Ss	0	Ss	Ss
	4127	Ss	Phantom Road	Ss	0	Ss	Ss
	4138	Sxs	Kinskuch	Ss	0	Sx	Ss
	4189	Sxs	Kitwanga	Sx	20	Sx	Ss
	4204	Sxs	Kildala	Ss	0	Ss	Ss
	7754	Sx	Knight Inlet	Ss	0	Ss	Ss
	7755	Sx	Knight Inlet	Ss	0	Ss	Ss
	7757	Sx	Knight Inlet	Sx	<5	Ss	Ss
	7761	Sx	Knight Inlet	Ss	0	Ss	Ss
	8602	Se	Purden Mountain	Si	100	Si	Si
	9963	Se	Dice Creek	Ss	0	Ss	Ss
	9964	Se	Dorothy Creek	Sx	<5	Ss	Ss
	9966	Se	Dice Creek	Ss	0	Ss	Ss
	28851	Sxs	Cranberry	Sx	10	Ss	Ss
	29201	Sxs	Kitwanga Road	Sx	60	Sx	Ss
Seedling	6333	Ss	Yellow Point (seed orchard)	Ss	0		
	4144	Sw	Tacheeda Mountain	Si	100		
	9833	Sxs	Whistler	Sx	54		
	28851	Sx	Cranberry	Sx	10	Ss	Ss
	28852	Sx	Calvin Creek	Sx	<5		
	29201	Sx	Kitwanga Road	Sx	34	Sx	Ss

[1]) Ss, Sitka spruce; Sw, white spruce; Se, Engelmann spruce; Si, interior spruce (white or Engelmann); Sx, hybrid spruce; Sxs, hybrid spruce with Sitka parentage.

by ligation of *BamHI* digested cpDNA with *BamHI* digested pUC8 followed by transformation into *E. coli* strain DH5α. Approximately 300 transformants containing inserts were selected as white colonies on LB plates containing ampicillin 100 μg/ml, 1% X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). Southern blotting and colony hybridization was carried out using Gene Screen filters following standard procedures (MANIATIS et al., 1982). Prehybridization, hybridization and washing of the filters was carried out at 65 °C as follows: Prehybridization was carried out in 6x SSPE, 1% sodium lauroyl sarcosine, 100 μg/ml sheared and denatured calf thymus DNA. The same solution was used for hybridization with the addition of 10% dextran sulphate and 3-5x 10^6 cpm of probe. The filters were then washed in 2xSSC, 0.1% SDS at 65 °C.

Results

Identification and cloning of chloroplast probes for distinguishing Sitka and interior spruce species

Electrophoresis of *BamHI* digested cpDNA from mature individuals revealed a 10.5 kb fragment unique to white spruce and fragments of 5.5 kb and 4.3 kb unique to Sitka spruce. The unique 10.5 kb *BamHI* fragment of white spruce was hybridized to the chloroplast and nuclear DNA fractions of each species. This showed that the unique Sitka *BamHI* fragments of 4.3 kb and 5.5 kb were homologous to this probe (*Figure 1A*). Also, it illustrated that fragments prepared from electrophoresis gels were not suitable for screening total DNA samples because of contamination with nuclear repetitive sequences which cause background problems (*Figure 1A*). The isolated 10.5 kb white spruce cpDNA fragment was used as a probe to screen the *BamHI* library of Sitka spruce cpDNA in pUC8. Clones containing each hybridizing fragment, pSS4 and pSS6, were recovered from the library. Inserts from each plasmid were then used as probes in hybridization with the nuclear and cpDNA of each spruce species (*Figure 1B and C*). These results showed that either probe may be used in distinguishing the species and that no hybridization is observed with the nuclear fractions.

Analysis of spruce seedlots

We chose to use the smaller probe, pSS4, to develop routine screening of hybrid seedlots. One hundred operationally produced seedlings from each of six seedlots were obtained from the B.C Ministry of Forests nursery at the end of the growing season. Needle samples (0.5 g) of all 100 seedling were pooled and total DNA prepared. Two of the seedlots (6333 and 4144) had been classified as pure species whereas the other four were thought to be hybrids based on their growth in the nursery. The results of the cpDNA analysis confirmed these observations (*Figure 2*) and allowed us to quantify the amout of each species represented. It is clear that in pure seedlots from outside the areas of introgression (Sitka spruce from Vancouver Island and interior spruce from the Prince George area, *Figure 5*) there is no detectable polymorphism within species for the 100 individuals screened. Seedlings from seedlots collected from regions within the coastal mountains showed cpDNA fragments typical of both species (*Table 1* and *Figure 5*).

The hybridizing bands were scanned using a densitometer with area integration and a standard curve was generated from a series of mixtures of white spruce and Sitka spruce total DNA standards (*Figure 3*). Hybridization to ENGELMANN spruce was also quantified and found to yield similar results to white spruce. The detection limit of the procedure allows less than 5 in 100 of either Sitka or interior species to be detected. While representations of less than 5% can be visualized using longer exposures of the autoradiograms, quantification is not possible at this level (*Figure 3*). Subsequent analyses were carried out using two-week old germinants from pooled samples of 200 seeds from each seedlot. A summary of these results is presented in *table 1* and compared to analysis of these seedlots based on growth performance (WOODS, 1988). The species assignments made using the cpDNA probe are in good agreement with those estimated by growth habit. In the case of seedlot #29201, seedlings following lifting showed significantly less representation

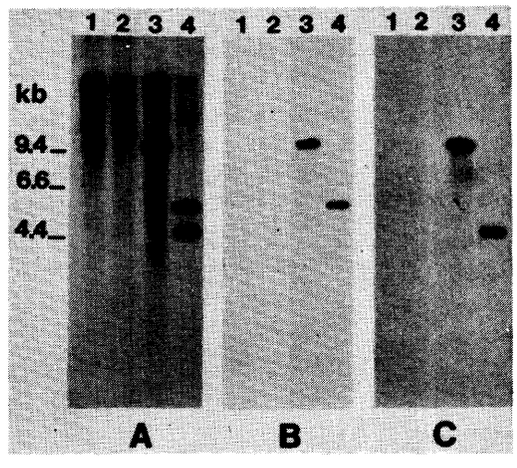


Figure 1. — Hybridization of spruce chloroplast probes to chloroplast and nuclear DNA of Sitka and white spruce. Lane 1, white spruce nuclear DNA; lane 2, Sitka spruce nuclear DNA; lane 3, white spruce chloroplast DNA; lane 4, Sitka spruce chloroplast DNA. All DNA samples were digested with *BamHI*. The probes used were as follows: Panel A, unique 10.5 kb *BamHI* cpDNA fragment of white spruce recovered from low melting point agarose gel; panel B, insert from homologous cp DNA clone (pSS6) of Sitka spruce; panel C, insert from homologous cp DNA clone (pSS4) of Sitka spruce.

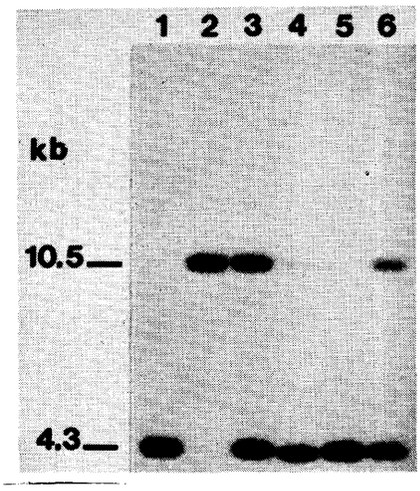


Figure 2. — Use of pSS4 as a probe for distinguishing coastal and interior spruce chloroplast DNA in seedlots. Total DNA was prepared from samples of 100 pooled seedlings from six seedlots (lanes 1 to 6). Lane 1, pure Sitka spruce #6633; lane 2, pure interior spruce #4144; lane 3, hybrid #9833; lane 4, hybrid #28851; lane 5, hybrid #28852; lane 6, hybrid #29201.

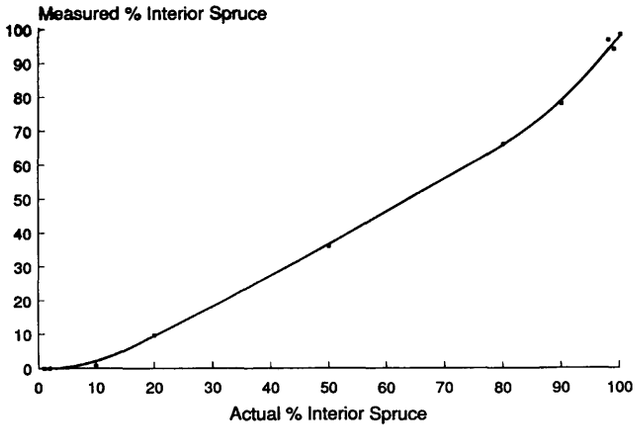


Figure 3. — Standard curve for determination of cpDNA content of interior and Sitka spruce. Measured percent of area of each peak for interior and Sitka spruce species specific bands was determined by densitometry and area integration. This was plotted against the percent of total DNA of each species applied to each lane.

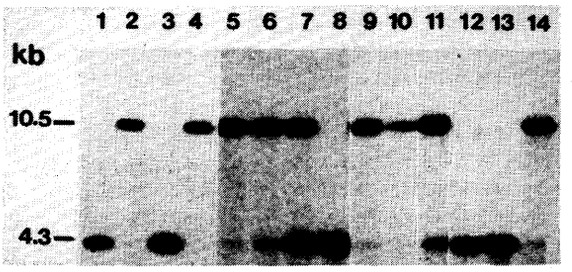
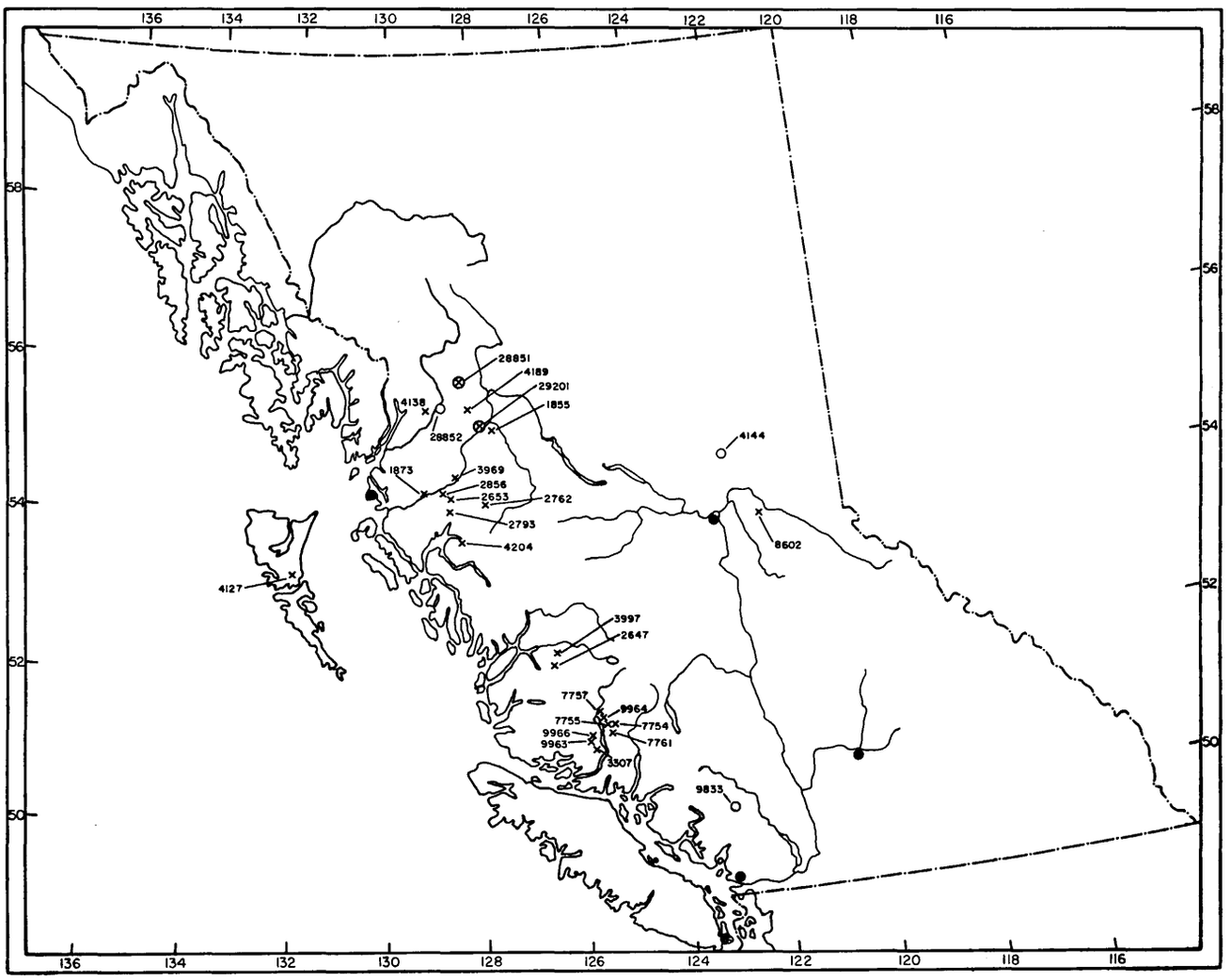


Figure 4. — Analysis of cp DNA content of individuals from seedlot #9833. DNA was isolated from the needles of 14 individuals digested with *BamHI* and Southern blotted. The blot was hybridized with the pSS4 insert.

of interior spruce than the pooled seed. This may be accounted for by the high culling rate of undersized seedlings grown from this seedlot, most of which are expected to be interior spruce. In the case of seedlot numbers 9963, 9964 and 9966 the species assignment Ss (Sitka spruce) made from the data of Woods and from the DNA data are both in disagreement with the tree seed registry entry Se (ENGELMANN spruce). This might be explained by the similar appearance of Sitka and ENGELMANN cones which would be hard to distinguish in wild seed collections. The distribution of hybrid and mixed seedlots (*Figure 5* and *Table 1)* shows that there is increasing representation of interior spruce as one moves from West to East across the coastal mountains.



Scale: 1: 10 144 927

Figure 5. **— Locations at which spruce seedlots were collected in British Columbia. x, seedlot used for analyses of seed; O, seedlot used for analysis of seedlings; seedlots used for both are marked with both symbols; ●, major cities.**

Analysis of individuals from hybrid seedlots

Analysis of seedlot #9833 from the Whistler region had shown about a 1:1 ratio of Sitka to interior spruce (*Figure 2*). We therefore chose to examine the cpDNA of individuals from this seedlot. Of 14 individuals examined 5 show the Sitka type cpDNA fragment, 6 show the interior spruce type DNA fragment and 3 individuals show a mixed cpDNA content (*Figure 4*). Although the parentage of these individuals is uncertain, biparental inheritance of chloroplasts is suggested.

Discussion

We have developed a reliable and convenient means of quantifying the species composition of spruce seedlots. Examination of one or two hundred individuals in several pure seedlots of Sitka and interior spruces indicates that no significant intraspecies variation occurs with respect to the cpDNA fragments used in the analysis.

Since the analysis can be performed using small amounts of tissue (approximately 0.5 g), it is possible to analyze seedlots within two weeks of germination. It should, therefore, be possible to analyze all seedlots prior to nursery growing or after cone collection and seed extraction; although, in the latter case one might expect error due to differential viability between species. This will enable growers to alter growing regimes to suit the predominant species or elect not to grow mixed seedlots, hence saving most of the loss now experienced. The analysis is in good agreement with the recommendations made for seedlots from the zone of introgression based on their nursery performance (Woods, 1988). The test described here should enable growing regimes to be adjusted without the need for prior assessment of the seedlings in a nursery.

The results using this probe quantify the proportion of interior spruce (Engelmann/white complex) but do not distinguish Engelmann and white spruce. While this distinction would be of interest to geneticists, it has no operational significance in nursery producton, since both species can be successfully grown under the same regime and are planted on similar sites.

The occurrence of both interior and Sitka chloroplast types in hybrid individuals is interesting. Analysis of cpDNA inheritance has been carried out for several gymnosperms including *Pinus* (Wagner et al., 1989; Neale and Sederoff, 1989), *Larix* (Szmidt et al., 1987) and *Pseudotsuga* (Neale et al., 1989). Inheritance is generally paternal. However, interspecific hybrids within both *Pinus* (Wagner et al., 1987) and *Larix* (Szmidt et al., 1987) may have the chloroplast types of both parents, or recombined chloroplasts. In controlled crosses between *L. decidua* and *L. leptolepis*, three of six progeny exhibited paternal inheritance, two exhibited biparental inheritance, and one exhibited maternal inheritance. Similar results are suggested for *Pinus banksiana* and *P. contorta* hybrids, but in this case frequent recombination (Wagner et al., 1987) and even segregation of chloroplast types is observed within individuals (Govindaraju et al., 1988). In addition, individuals heteroplasmic with respect to chloroplasts have been found in *Pinus monticola* (White, 1990). Based on our results *Picea* hybrids exhibit heteroplasmy, suggesting biparental inheritance. We did not, however, detect any recombined or novel restriction fragments among the hybrid individuals in the small number of samples analyzed.

Since the seedlots were collected from a number of individuals, it is not known whether their intermediate growth pattern is due to the existence of hybrids or a mixture of individuals within the seedlot. The occurrence of individuals with mixed cpDNA composition demonstrates that these seedlots do, in fact, contain hybrid individuals. It is not possible to determine the exact frequency at which hybrid individuals occur as we do not snow the frequency at which biparental inheritance occurs under controlled conditions. We are currently generating hybrid individuals from controlled crosses to examine this question.

Acknowledgements

This work was supported in part by the Science Council of British Columbia and the Forest Resource Development Agreement. The authors would also like to thank Gyula Kiss for providing needle samples for type trees and Kim Gowe for help in preparation of the manuscript.

Literature Cited

Brix, H.: Growth response of Sitka spruce and white spruce seedlings to temperature and light intensity. Can. For. Serv., Pac. For. Res. Centre., Inf. Rep. BC-X-74 (1972). — Daubenmire, R.: Taxonomic and ecological relationships between *Picea glauca* and *Picea sitchensis* and their ecological interpretation. Can. J. Bot. **46**: 787—798 (1968). — El-Kassaby, Y. A., Sigurgeirsson, A. and Szmidt, A. E.: The use of restriction analysis of chloroplast DNA in classifying hybrid spruce seedlots. In: Molecular Genetics of Forest Trees. Proceedings of the Frans Kempe symposium, Umei, June 14—16 1988. Ed. Jan-Erik Hallgren, Sveriges Lantbruksuniversitet, Institutionen for skoglig genetik ach vaxtfysiologi, Umea. pp. 67—88 (1988). — Feinberg, A. P. and Vogelstein,B.: A technique for radiolabelling of restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**: 6—13 (1983. — Feinberg, A. P. and Vogelstein, B.: Addendum to "A technique for radiolabelling of restriction endonuclease fragments to high specific activity". Anal. Biochem. **137**: 266—267 (1984). — Govindaraju, D. R., Wagner, D. B., Smith, G. P. and Dancik, B. P.: Chloroplast variation within individual trees of a *Pinus banksiana — Pinus contorta* sympatric region. Can. J. For. Res. **18**: 1347—1350 (1988). — Krajina, V. T., Klinka, K. and Worrall, J.: Distribution and ecological characteristics of trees and shrubs of British Coulmbia. U.B.C. Faculty of Forestry. 131 pp (1982). — Maniatis, T., Fritsch, E. F. and Sambrook, J.: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, NY (1982). — Neale, D. B., Marshall, K. A. and Sederoff, R. R.: Chloroplast and mitochondrial DNA are paternally inherited in *Sequoia sempervirens* D. Don Endl. Proc. Natl. Acad. Sci. USA **86**: 9347—9349 (1989). — Neale, D. B. and Sederoff, R. R.: Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. Theor. Appl. Genet. **77**: 212—216 (1989). — Neale, D. B., Wheeler, N. C. and Allard, R. W.: Paternal inheritance of chloroplast DNA in Douglas-fir. Can. J. For. Res. **6**: 1152—1154 (1986). — Roche, L.: A genecological study of the genus *Picea* in British Columbia. New Phytol. **68**: 505—554 (1969). — Szmidt, A. E., Alden, T. and Hallgren, J.-E.: Paternal inheritance of chloroplast DNA in *Larix*. Plant Mol. Biol. **9**: 59—64 (1987). — Szmidt, A. E., El-Kassaby, Y. A., Sigurgeirsson, A., Alden, T., Lindgren, D, and Hallgren, J.-E.: Classifying seedlots of *Picea sitchensis* and *P. glauca* in zones of introgression, using restriction analysis of chloroplast DNA. Theor. Appl. Genet. **76**: 841—845 (1988). — Wagner, D.B., Furnier, G. R., Saghai-Maroof, M. A., Williams, S. M., Dancik, B. P. and Allard, R. W.: Chloroplast DNA polymorphism in lodgepole and jack pines and their hybrids. Proc. Natl. Acad. Sci. U.S.A. **84**: 2097—2100 (1987). — Govindaraju, D. R. Wagner, D. B. Yeatman, C. and Pitel, J. A.: Paternal chloroplast DNA inheritance in a diallel cross of jack pine (*Pinus banksiana* Lamb.). J. Hered. **80**: 483—485 (1989). — White, E. E.: A method for extraction of chloroplast DNA from conifers. Plant Mol. Bio. Rep. **4**: 98—101 (1988). — White, E. E.: Chloroplast DNA in *Pinus monticola*. 2. Survey of within species variability and detection of heteroplasmic individuals. Theor. Appl. Genet. **79**: 251—255 (1990). — Woods, J. H.: Sitka-interior hybrid spruce seedlot trials. Unpublished Report to BC Ministry of Forests. Aug. 8th 1988 (1988).