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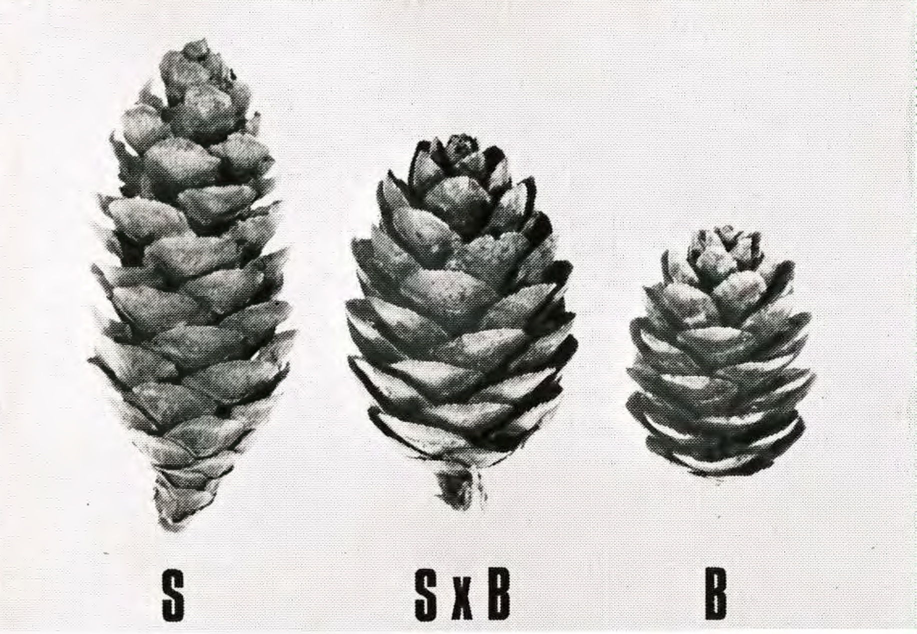

HYBRIDIZATION OF BLACK SPRUCE AND SERBIAN SPRUCE

by
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MARITIMES

CANADIAN FORESTRY SERVICE

MARITIMES FOREST RESEARCH CENTRE

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ABSTRACT

From 1964 to 1974 the cross black spruce X Serbian spruce, or reciprocal, was attempted on 23 trees. All but three of the attempted crosses yielded full seeds. These seeds were used to establish nine separate greenhouse, nursery, and field experiments.

Crossability of black spruce X Serbian spruce is estimated as 0.40 and the reciprocal as 0.62 (overall mean = 0.44). Accepting crossability as indicative of degree of relationship, black and Serbian spruce bear the closest relationship between Old World and New World spruces demonstrated to date. Black spruce is more closely related to Serbian spruce than it is to any New World spruce. The phylogenetic relationship of black and Serbian spruce is discussed.

All but one hybrid population exhibits heterosis in the statistical sense (exceeds the mid-parent value). In greenhouse and nursery tests and in field tests up to 5 years from planting, the hybrids perform about as well as the best parent, black spruce. In trials older than 5 years from field planting, the hybrids are consistently taller and survive equally as well as the best parent, black spruce. Means for mass producing the hybrid are discussed.

RESUME

De 1964 à 1974 le croisement simple ou réciproque de l'Epinette noire avec l'Epinette de Serbie a été tenté sur 23 arbres. Tous ces croisements, sauf trois, ont produit des graines pleines. Ces graines ont été utilisées pour établir neuf expériences en serre, en pépinière et en plein champ.

L'hybridabilité de l'Epinette noire avec l'Epinette de Serbie est estimée à 0.44 (simple) et 0.62 (réciproque), la moyenne globale étant 0.44. En admettant l'hybridabilité comme indice du degré de parenté, l'Epinette noire et l'Epinette de Serbie affichent la plus étroite parenté démontrée jusqu'à présent entre les épinettes du Nouveau Monde et de l'Ancien Monde. L'Epinette noire est plus apparentée à l'Epinette de Serbie qu'à n'importe quelle épinette du Nouveau Monde. La relation phylogénétique de l'Epinette noire et de l'Epinette de Serbie est discutée.

Hormis une exception, toute la population hybride accuse de l'hétorisis au sens statistique (excède la valeur de l'un et l'autre parent). Jusqu'à 5 ans après la plantation dans les expèriences en serre, en pépinière et en plein champ, les performances des hybrides sont presque aussi bonnes que celles du meilleur parent, l'Epinette noire. Dans les essais remontant à plus de 5 années depuis la plantation en plein champ, la hauteur des hybrides est supérieure et leur survie égale à celles du meilleur parent, l'Epinette noire. Les moyens de production massale de l'hybride sont discutés.

INTRODUCTION

Species hybridization is a technique used by tree breeders to create new genetic combinations that are not available in nature and there is a possibility that the hybrids may have economically important traits that are superior to the parental species.

Hybridization has also been used to help determine phylogenetic relationships within tree genera (Critchfield 1973, Wright 1955).

The author has been involved with species hybridization studies in the genus *Picea* since the mid-1960's. One of the most promising hybrids produced during this period is *P. mariana* (Mill.) B.S.P. X *P. omorika* (Pancic) Purkyne (black spruce X Serbian spruce) and the reciprocal.[1] In this paper the results of several experiments designed to produce and test these hybrids are reported.

Serbian spruce is a relict species confined to a small area (25 X 25 km) of the Dinaric Alps of Yugoslavia where it is found in small isolated stands on limestone soils at an elevation of 1000 to 1750 m. The tree is slender with short ascending branches and attains a height of 30 m and occasionally 40 m (Schmidt-Vogt 1977). The species is not exploited in its natural habitat but has shown some promise as an exotic in northern Europe and Britain where it is considered to be exceptionally hardy to wind, frost, and drought and is unexacting in its site requirements (Streets 1962). Serbian spruce is generally recognized as rather slow-growing but has attained a growth rate (height) of 0.6 m/yr in southern England and 0.3 m/yr in northeastern Scotland (Streets 1962). The species has been planted on a very limited scale in eastern North America where it has not excelled except possibly as an ornamental.

Black spruce is a transcontinental North American species. Its range extends from coastal Alaska to Newfoundland and from near the Arctic Ocean to central Pennsylvania. The species is a slender, shallow rooted, small to medium size tree capable of attaining a height of 20 m, and occasionally 30 m, on good upland sites. In the absence of disturbance, black spruce is usually restricted to cool wet sites over much of the southern part of its range, but further north, the species occupies a wide range of sites.

Black spruce is the most widely planted tree species in eastern Canada (Morgenstern and Carlson 1979). It is an early successional species, well adapted to planting on exposed sites. The species is easy to handle in the nursery and exhibits good early growth over a wide range of planting sites (Fowler and Coles 1979). Black spruce is also considered to be less susceptible to spruce budworm, *Choristoneura fumiferana* (Clemens) than other spruces native to eastern Canada.

A possible drawback to the extensive planting of black spruce is that it is a relatively short-lived species that does not attain large dimensions. Thus, it is usually planted as a fibre producer and the forest manager does not have the option of postponing the harvest in order to produce other products, such as sawlogs.

Among taxonomists there is a lack of consensus on how the genus *Picea* should be subdivided. For a detailed review of this subject the reader is referred to Schmidt-Vogt (1977). Most of the early taxonomists divide the genus into three sections: Eupicea (or Morinda), Casicta, and Omorika. Black spruce is assigned to section Eupicea and Serbian spruce to section Omorika.

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[1] By convention the female parent of a hybrid is listed first e.g., Serbian spruce is the female parent and black spruce the male parent of the hybrid Serbian X black.

Wright (1955) in his paper "Species crossability in spruce in relation to distribution and taxonomy" suggests that there is no natural break in the genus sufficient to warrant the erection of section lines. However, he considers Serbian and black spruce to be only distantly related. More recently Mikkola (1969), based on studies of interspecific sterility, suggested the division of the genus into two sections or groups (Abies and Omorika). He is the first to suggest that Serbian and black spruce are closely related and that they should be placed in the same group (Omorika). Schmidt-Vogt (1977) also recognizes these two distinct groups with white spruce, *P. glauca* (Moench) Voss, assigned as intermediate between the groups. However, he does not attempt to assign *P. mariana* to either group.

Oksbjerg (1953) in Denmark, was the first to report a cross of Serbian and black spruce. He did not provide information on species crossability or performance of the hybrid in relation to the parental species. Mention is also made of the hybrid black X Serbian spruce (*P. mariorika*) by Boom (1959, in Schmidt-Vogt 1977). Another attempt to cross these two species was made at the Institute of Forest Genetics, Rhinelander, Wisconsin in 1959 (Jeffers 1971). Again, no information is available on crossability, however, at age 8 years (from seed) the hybrids were clearly superior in height to black spruce.

Starting in the mid-1960's several successful attempts to cross Serbian and black spruce have been reported. Full seeds were obtained from the cross, black X Serbian by Fowler (1966, 1968), Holst (1966), Mikkola (1969), Rauter (1970, 1971a, 1973), Gordon (1973, 1976) and Nienstaedt (1977), and from the reciprocal cross by Fowler (1966), Rauter (1973, 1975), Gordon (1973, 1976), and Nienstaedt (1977). Gordon (1976) reported black and Serbian spruce to have a crossability of 48-71%, which corresponds closely to Nienstaedts' (1977) estimates of 50-84%, and is indicative of a close phylogenetic relationship.

Nienstaedt (1977) suggested that, because of the high crossability and similarity of flower phenology, hybrids of black and Serbian spruce could be mass produced in clonal seed orchards.

Rauter (1976) reported the hybrids black X Serbian and reciprocal to be heterotic in nursery tests in Ontario. In these tests the black X Serbian seedlings were superior to seedlings derived from the reciprocal cross.

MATERIALS AND METHODS

During 1964-1974, several attempts were made to cross black and Serbian spruce at Maple and Midhurst, Ontario (1964, 1965), Petawawa National Forest Institute (PNFI) (1967) and at the Acadia Forest Experiment Station (AFES) near Fredericton, N.B. (1969, 1971, 1972, and 1974). Except for the 1967 pollinations, which were made by Mark Holst (PNFI), all pollinations were carried out under the direction of the author. The techniques used for controlled pollination are essentially those reported by Nienstaedt and Teich (1971). A summary of pollinations attempted during the period 1964 to 1974 is presented in Table 1.

The general procedures used for testing the hybrids were as follows. The seeds were placed on moist coarse sand in petri dishes and stored for 3 to 10 days at 5°C in a refrigerator. The seeds were then germinated at 22°-25°C and transplanted into a greenhouse in individual 250-300 cc pots containing a 1:1:1 (sand:peat: humus) mix. For experiments started in the winter, the seedlings were

Table 1. Summary of controlled pollinations with black and Serbian spruces, 1964-1974

Pollination year	Female Parent			Male Parent			Pollen year	Population number	Experiment number
	Species	Identification number	Location	Species	Identification number	Origin			
1964	Black	13 trees	Maple	Black	Mix	Maple	1964	-	-
	Black	S 42	Maple	Serbian	S 20	Midhurst	1964	SP 58	82
	Black	S 51	Maple	Serbian	S 20	Midhurst	1964	SP 63	82
	Black	S 52	Maple	Serbian	S 20	Midhurst	1964	no full seeds	
	Black	S 56	Maple	Serbian	S 20	Midhurst	1964	SP 67	82
1965	Serbian	S 29	Midhurst	Serbian	S 31	Midhurst	1965	1193	80
	Serbian	S 29	Midhurst	Serbian	S 29	Midhurst	1965	1194	80
	Serbian	S 31	Midhurst	Serbian	S 29	Midhurst	1965	1196	80
	Serbian	S 31	Midhurst	Serbian	S 31	Midhurst	1965	1197	80
	Serbian	S 29	Midhurst	Black	Mix	PNFI	1965	1195	80
	Serbian	S 31	Midhurst	Black	Mix	PNFI	1965	1198	80
1967	Black	ST 1792	PNFI*	Serbian	P 1614	Rochester	1967	1610	100,134
	Black	ST 1794	PNFI	Serbian	P 1620	Maple	1967	1615	100,134
	Black	ST 1795	PNFI	Serbian	P 1626	PNFI	1967	1619	100,134
1969	Black	ST 566	AFES**	Black	196	AFES	1969	-	-
	Black	ST 566	AFES	Serbian	Mix	Midhurst	1968	2191	105
1971	Black	6 trees	AFES	Black	Mix	AFES	1971	-	-
	Black	71-20	AFES	Serbian	Mix	Midhurst	1969	2215	118
	Black	71-21	AFES	Serbian	Mix	Midhurst	1969	2216	118

Table 1. Cont'd

Pollination year	Female Parent			Male Parent			Pollen year	Population number	Experiment number
	Species	Identification number	Location	Species	Identification number	Origin			
1972	Black	11 trees	AFES	Black	Mix	AFES	1972	-	-
	Black	72 A	AFES	Serbian	Mix	Finland	1971	2403	-
	Black	72 B	AFES	Serbian	Mix	Finland	1971	2404	-
	Black	72 C	AFES	Serbian	Mix	Finland	1971	2405	123
	Black	72 D	AFES	Serbian	Mix	Finland	1971	2406	123
	Black	72 E	AFES	Serbian	Mix	Finland	1971	2407	123
	Black	72 F	AFES	Serbian	Mix	Finland	1971	no full seeds	
	Black	72 G	AFES	Serbian	Mix	Finland	1971	2408	123
	Black	72 H	AFES	Serbian	Mix	Finland	1971	no full seeds	
1974	Black	ST 566	AFES	Black	Mix	AFES	1974	2709	138,141
	Black	ST 566	AFES	Serbian	Mix	Rhinelander	1973	2706	138,141
	Black	ST 1132	AFES	Black	Mix	AFES	1974	2712	138,141
	Black	ST 1132	AFES	Serbian	Mix	Rhinelander	1973	2711	138,141
	Black	ST 1133	AFES	Black	Mix	AFES	1974	2717	138,141
	Black	ST 1133	AFES	Serbian	Mix	Rhinelander	1973	2714	138,141

* Petawawa National Forest Institute.

** Acadia Forest Experiment Station.

Site Codes

very dry ← Moisture Regime → very wet

Richness		0	1	2	3	4	5	6	7	8	9
very rich	1					1					
	2				2		3				
	3			4		5		6			
	4		7		8		9		10		
very poor	5	11		12		13		14		15	

These codes indicate mixedwood succession (normal ecoclimate)

Codes 20 units higher than in triangle indicate coniferous succession (cooler ecoclimates)

Codes 40 units higher than in triangle indicate hardwood succession (warmer ecoclimates)

Fig. 1. A classification for forest sites on the Acadia Forest Experiment Station, Loucks, O.L. 1957. Site classification during 1957 at Acadia and Green River. Can. Dep. North. Aff. Nat. Resour. For. Br., Marit. Dist. File Rep. 186 Mimeo.

nursed under an 18 h photoperiod at 20°-25°C during the light period and 16°C during the dark period. When the seedlings were 5-6 months of age they were transplanted into nursery beds at 15 X 15 cm spacing.

After two growing seasons in the nursery the seedlings were transplanted to one or more field trials at AFES or elsewhere in the Maritimes Region. All planting at AFES was done on recently cleared forest land from which logging debris had been removed. A forest site classification for AFES[2] based on moisture, richness, and normal successional trends is presented in Fig. 1 and is used throughout this paper to help describe the planting sites used at AFES.

Growth data were subjected to analysis of variance and mean separation was carried out using the Scott-Knott cluster analysis method (Gates and Bilbro 1978).

1964 Pollinations - Experiment 82

In spring 1964, as part of a larger exploratory interspecific crossing program in *Picea*, four black spruce trees of Thunder Bay, Ontario origin, growing at the Southern Research Station, Maple, Ontario were pollinated with Serbian spruce pollen from a single tree. In addition, intraspecific crosses were made on 13 black spruce trees of the same origin. In December 1965, seeds from these crosses were germinated and transplanted into a greenhouse at Maple, Ontario. In mid-January, 1966, when the seedlings were approximately 2 weeks old, the number of cotyledons was recorded and hypocotyl color was rated on a scale of 0-4.

0 = no red pigment (Munsell (1929)) 2.5 GY 6/8
2 = some red pigment (Munsell (1929)) 10 R 5/8
4 = lower half of hypocotyl red (Munsell (1929)) 5 R 5/8

Total height of all seedlings was recorded in March when the seedlings were about 10 weeks old.

In July 1966, the seedlings were shipped to Fredericton, N.B. and planted in the AFES nursery. Seedling height was measured at the end of the 1967 growing season. In May 1969, the seedlings were planted at 3 X 3 m spacing in a randomized single tree plot design at AFES. The planting site was a recent clearcut which formerly supported a mixed wood cover of white birch, red maple, black and white spruce. The site (site 24) is classified as moderately fertile and moderately fresh and would normally follow a coniferous succession. Seedlings from a local AFES origin were planted as additional controls and as surrounds for the plantation. Ten-year (from planting) height and survival were recorded in October 1978.

1965 Pollinations - Experiment 80

In spring 1965, controlled pollinations were attempted on two Serbian spruces growing at Midhurst, Ontario. Each tree received the following pollens: cross (pollen from the other tree), self (pollen from the same tree) and black spruce (pollen-mix from several trees from PNFI).

Experiment 80A In mid-December 1965, 100 full seeds from each of the controlled crosses and seeds from open pollinated PNFI black spruce were germinated and nursed as for experiment 82. The experimental design in the greenhouse was randomized 12-seedling row plots replicated up to eight times. When the seedlings were about 2 weeks old, the number of cotyledons was recorded and hypocotyl color rated as in experiment 82.

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[2] Loucks, O.L. 1957. Site classification during 1957 at Acadia and Green River. Can. Dept. North. Aff. Nat. Resour., For. Br., Maritime Dist. File Rep. 186. Mimeo.

Total height was recorded when the seedlings were 9, 12, 16, 21 and 26 weeks old. In July 1966, the seedlings were shipped by air freight, to Fredericton, N.B. and transplanted into the AFES nursery. The nursery design was randomized 6-tree row plots, replicated seven times. Total height was recorded at the end of the 1968 growing season.

In May 1969, the seedlings were field planted into a randomized 2-tree plot design replicated 14 times at 3 X 3 m spacing. Seedlings of an AFES black spruce provenance were used as controls and for a single row of surrounds for the plantation. The plantation site was similar to that described for experiment 82.

Survival and total height were recorded at the end of the 1970, 1972, and 1978 growing seasons when the trees were 2, 4 and 10 years from planting. Diameter breast height was recorded in 1978.

Experiment 80B In December 1966, 50 full seeds from each of the same seed lots used in Experiment 80A, plus one additional open-pollinated AFES black spruce seed lot were germinated and transplanted into a greenhouse experiment at AFES in much the same manner as described for experiments 82 and 80A except that the greenhouse design was randomized 8-seedling row plots, replicated five times. Seedling height was recorded when the seedlings were 28 weeks old.

The seedlings were transplanted to the AFES nursery at the end of July 1967 into randomized 6-tree row plots replicated six times. Seedling height was measured at the end of the 1968 and 1969 growing seasons. The seedlings were transplanted under the direction of Mr. D. Levy, Nova Scotia Department of Lands and Forests, into a field experiment near Lawrencetown, N. S., in the spring of 1970. Planting was at 3 X 3 m spacing in a randomized 4-tree square plot design replicated six times. The planting site was an old field which formerly supported hay. The soil was an imperfectly drained heavy clay loam. Survival, height, and diameter breast height were recorded at the end of the 1976 growing season (7 years from planting) by D. Levy.

Experiment 80C In December 1965, 100 full seeds from each of the six crosses made at Midhurst, Ontario were sent to Mark Holst (PNFI). These seeds, along with those of several other spruce hybrids (including two black X Serbian spruce hybrids) were sown in a greenhouse in March-April 1966. Morgenstern (1973) provides a brief description of this test at PNFI. The seedlings were transplanted into the PNFI nursery in 1967 and field planted in June 1968. The field design was a modified randomized 7-tree row plot design, replicated four times at 1 X 1 m spacing. Black spruce from Petawawa, Fort William X Petawawa, Notakin Depot, P.Q. X Petawawa, and Kapuskasing, Ontario were included as controls. The planting site, adjacent to the PNFI nursery, was a well-drained, moderately fertile sandy soil. Dead seedlings were replaced in November 1969.

Morgenstern (1973) summarized the results of the 1972 (5 years) height measurements. At that time, the smallest seedlings were selfed Serbian spruce (mean 70 cm) and the tallest were black X Serbian hybrids (168 cm). The plantation was again measured by Morgenstern at the end of the 1978 growing season (11 years).

1967 Pollinations - Experiments 100 and 134

In the spring 1967, Mark Holst, PNFI, pollinated three black spruce trees, one from each of Dog River, Kapuskasing, and PNFI, Ontario with Serbian spruce pollen from Rochester, N.Y., Maple, and PNFI, Ont., respectively. Cones from these crosses were sent to Fredericton where the seeds were extracted and used to establish greenhouse, nursery, and field trials.

Experiment 100 Two hundred full seeds from each of the three hybrid populations, from two populations of Serbian spruce from natural stands in Yugoslavia, and one population of black spruce from Green River, N.B. were germinated and planted in a greenhouse experiment in July 1969 and transplanted into the nursery in June 1970. The procedures used were essentially the same as those described for experiment 80 except that the experimental design in the greenhouse was randomized 5-tree row plots replicated eight times with extra germinants planted in unreplicated plots. The design in the nursery was 6-tree row plots replicated 10 times.

In June 1972, three field experiments were established. In each experiment the trees were planted into 4-tree square plots at 2.4 X 2.4 m in a randomized block design replicated six times. The site for plantation 100A, AFES, is classified as dry and infertile and would normally support a conifer cover (site 27). Plantation 100B, also at AFES, is moderately dry and moderately rich and would normally support a conifer cover (site 24). Plantation 100C was planted by K. Roller in Queens County, N.S. on land owned by Bowater-Mersey Paper Company. This site is rated as moderately fresh and moderately rich and would normally support a mixedwood overstory.

Total height of all surviving seedlings in the three tests was recorded in the fall 1977 at the end of the sixth growing season from planting.

Experiment 134 In late February 1974, extra seeds from the 1967 pollination were germinated and planted in the greenhouse. The seedlings were transplanted into the nursery in late July of the same year. No greenhouse or nursery measurements were made. In spring 1977, 20 seedlings from each of the three hybrid populations, two Serbian spruce populations, and a local black spruce population were field planted at AFES. The experimental design was randomized single-tree plots. The planting site is classed as moderately moist and rather infertile and would normally follow a coniferous succession (site 28). Low wet depressions are scattered throughout the planting area. Total height was measured for all surviving seedlings at the end of the 1979 growing season when the trees were two years from planting.

1969 Pollinations - Experiment 105

In spring 1969, a 3-tree Serbian spruce pollen mix from Midhurst, Ontario was applied to the strobili of one black spruce growing at AFES.

In June 1970, seeds of the hybrid population, two populations of Serbian spruce from Yugoslavia, and one population of AFES black spruce were germinated and planted in a greenhouse. The seedlings were overwintered in a shadehouse and transplanted to the AFES nursery in June 1971. In spring 1973, two small field trials were established at AFES. The seedlings were planted at 2 X 2 m spacing in randomized 4-tree square plots replicated four times. Experiment 105A was established on a fresh, moderately rich site (site 4) whereas 105B was planted on a dry, infertile site (site 7).

Total height of all surviving trees was measured in August 1978, 6 years from planting.

1971 Pollinations - Experiment 118

Two young black spruce of local AFES origin were pollinated with 2-year-old pollen from one Serbian spruce from Midhurst, Ontario. Six additional black spruce of the same local origin were pollinated with a local black spruce 5-tree pollen mix as control.

Seeds from the two hybrid populations, two Serbian spruce populations from Yugoslavia, and one black spruce population from AFES were germinated and transplanted into the greenhouse in January 1972. In June, when the seedlings were six months old and just prior to transplanting into the AFES nursery, up to 20 seedlings from each population were measured for total height and needle length. Observations were recorded for needle serrulations, stem form, foliage color, and number of cotyledons. The hybrid population 2216 had insufficient seedlings for further testing. The experimental design in the nursery was randomized 6-tree row plots replicated six times. Total height for all surviving seedlings was measured at the end of the 1973 growing season.

The hybrid seedlings and parental controls were field planted in two locations at AFES in the spring of 1974. Experiment 118A was planted on a moderately fresh, moderately fertile site (AFES site 4) whereas 118B was on an imperfectly drained less fertile site (AFES site 8). The experimental design for both tests was randomized 4-tree square plots replicated four times. Total height of all surviving trees was measured at the end of the 1978 growing season when the seedlings were five years from planting.

1972 Pollinations - Experiment 123

In spring 1972, eight black spruce trees growing at AFES were pollinated with a 9-tree Serbian spruce pollen mix from Finland. The Serbian spruce pollen was 1-year-old and evidently had deteriorated in transit or in storage as in vitro germination was poor. Controlled pollination of 11 additional AFES black spruce with a local black spruce pollen mix served as controls. A few full seeds were obtained from six of the eight hybrid crosses. Seeds from these six populations, one population of Serbian spruce from Yugoslavia, and one population of local black spruce were germinated and transplanted to the greenhouse in early February 1973. The single full seed from hybrid population 2404 failed to germinate. In late July, when the seedlings were about six months old, and just prior to transplanting to the nursery, total height and needle length were measured and observations on needle color and stem form were recorded. The experimental design in the nursery was randomized 6-tree row plots replicated up to six times depending on number of seedlings available. Total height of all surviving seedlings was measured in the nursery at the end of the second growing season (1975). No seedlings of population 2403 were alive at that time.

The seedlings were field planted at AFES in May 1976. The planting site was moderately dry and moderately fertile and would normally follow a coniferous succession (site 24). The test design was randomized single tree plots. Total height was measured at the end of the 1978 growing season when the trees were three years from planting.

1974 Pollinations - Experiments 138 and 141

Experiment 138 In spring 1974, three black spruce trees growing at AFES were pollinated with a mix of pollen from five Serbian spruce trees growing at Rhinelander, Wisconsin and a 5-tree black spruce pollen mix from AFES. Fifty seeds from each of these six families, as well as seeds from open-pollinated Serbian spruce populations, were germinated and transplanted into the greenhouse in January 1976. In July when the seedlings were approximately 6 months old and just prior to transplanting to the AFES nursery, total height was recorded. Total height was again measured in September 1977 at the end of the second growing season in the nursery. In spring 1978, the seedlings were planted into field trials at AFES and near Liverpool, N.S. No further measurements have been made to date.

Experiment 141 In January 1978, 100 additional seeds per family (except MS2714 which had only 58 seeds) were germinated and transplanted into the greenhouse. Total height was recorded for 20 seedlings from each family in July just prior to transplanting into the nursery. In October 1979 at the end of the second growing season in the nursery, total height was recorded for all surviving seedlings.

Hybrid verification

In addition to the measurements and observations on hypocotyl color and number of cotyledons (exp. 80, 82 and 118), in 1977 Miss Judy Loo, then a student at the University of New Brunswick, working under the direction of the author, carried out a phenetic study of the Serbian, black, and hybrid spruces growing at AFES (exp. 80, 82, 100, 105, 118, 123). She studied foliage and cones (when available) from five Serbian spruce populations (represented by 49 trees), four black spruce populations (43 trees) and 14 hybrid families (90 trees). Using methods described in detail by Harrison and Valentine (1972), Miss Loo calculated Character State Distances (CSD) for 16 characters of the parental species and Affinity Indices (AI) for the putative hybrids. CSD is an index of taxonomic differences which provides a measure of the reliability of a trait in distinguishing between two species. AI is a measure of the phenotypic similarity of an unknown tree to the reference populations. The characters studied are listed in Table 6.

RESULTS

The results of the 1964-1974 controlled pollinations are presented in Table 2. All but three of the 23 crosses attempted between black and Serbian spruce were successful. Numbers of full seeds per cone for the hybrids ranged from 0 to 6.2 ($\bar{x}$ = 2.1) for black X Serbian, 24.2 to 41.8 ($\bar{x}$ = 33.0) for Serbian X black, and for the parental species from 3.1 to 19.4 ($\bar{x}$ = 9.5) for black, 30.8 to 94.9 ($\bar{x}$ = 62.6) for crossed Serbian, and 30.7 to 49.0 ($\bar{x}$ = 39.9) for selfed Serbian spruce.

Seed Germination, Growth, and Survival

Pertinent data on germination and seedling growth in the greenhouse, nursery, and field are presented in Tables 3 and 4. More detailed information on early seedling performance is presented graphically in Fig. 2.

Experiment 80 Weight of the hybrid and selfed Serbian spruce seed, as expected, was essentially the same as that of non-hybrid, crossed seed of the same maternal parent (3.2 mg). The black spruce seed was only about one-third the weight of the Serbian spruce seed (1.1 mg). Differences in total germination were not significant; however, 6% of the hybrid seeds had abnormal germination (reverse embryos or double embryos). None of the pure Serbian spruce seeds and only one black spruce seed (0.7%) germinated abnormally.

Table 2. Summary of results of controlled pollinations with black (B) and Serbian (S) spruces

Pollination year	Experiment number	Population number	Controlled cross	No. of cones	No. of full seeds	No. of full seeds per cone	Crossability[1] %
1964	$\bar{x}$ 13 trees		B X B	437	1934	4.4	
	82	SP 58	B X S	17	49	2.9	65.9
	82	SP 63	B X S	30	3	0.1	2.3
	82	-	B X S	11	0	0	0
	82	SP 67	B X S	5	4	.8	18.1
1965	80	1193	S X S	10	944	94.4	
	80	1194	S-self	10	490	49.0	
	80	1196	S X S	17	518	30.8	
	80	1197	S-self	23	707	30.7	
	80	1195	S X B	37	1548	41.8	44.3
	80	1198	S X B	91	2206	24.2	78.6
1967	100,134	1610	B X S	242	1264	5.2	
	100,134	1615	B X S	52	288	5.5	
	100,134	1619	B X S	178	353	2.0	
1969	1 tree		B X B	48	149	3.1	
	105	2191	B X S	6	37	6.2	200.0
1971	$\bar{x}$ 6 trees		B X B	120	2326	19.4	
	118	2215	B X S	53	157	3.0	15.5
	118	2216	B X S	25	8	0.3	1.5

Table 2. Cont'd

Pollination year	Experiment number	Population number	Controlled cross	No. of cones	No. of full seeds	No. of full seeds per cone	Crossability %
1972	$\bar{x}$ 11 trees		B X B	284	2883	10.2	
		2403	B X S	106	2	0.02	0.1
		2404	B X S	34	1	0.03	0.3
	123	2405	B X S	22	9	0.41	4.0
	123	2406	B X S	298	17	0.06	0.6
	123	2407	B X S	388	29	0.07	0.7
		-	B X S	69	0	0	0
	123	2408	B X S	86	69	0.80	8.0
		-	B X S	83	0	0	
1974	138,141	2709	B X B	49	655	13.4	
	138,141	2706	B X S	67	325	4.9	36.6
	138,141	2712	B X B	170	611	3.6	
	138,141	2711	B X S	198	391	2.0	55.6
	138,141	2717	B X B	181	2214	12.2	
	138,141	2714	B X S	108	108	1.0	8.2

[1]

$$\text{Crossability (\%)} = \frac{\text{number of full seeds per cone interspecific}}{\text{number of full seeds per cone intraspecific}} \times 100$$

Table 3. Experiment 80 Germination and early growth of black (B), Serbian (S), and hybrid spruces

Exp.	Population number	Species or hybrid	Germination % Total	Germination % Normal	Greenhouse 6 month Height[1] cm	Nursery 2 year Height cm	Plantation 2 year Height cm	Plantation 4 year Height cm	Plantation 4 year Survival %	Plantation 8 year Height m	Plantation 8 year Dbh cm	Plantation 8 year Survival %
80A	1193	S X S	97	97	16 b	33 c	46 c	94 b	71	2.4 b	3.0 b	25
	1194	S(self)	95	95	13 b	31 c	37 d	72 c	82	1.0 c	- c	14
	1195	S X B	89	83	20 a	44 b	62 b	145 a	93	3.6 a	4.4 a	50
	1196	S X S	97	97	15 b	32 c	45 c	82 b	75	2.0 b	2.1 b	14
	1197	S(self)	97	97	14 b	27 d	35 d	57 c	71	1.8 b	2.1 b	7
	1198	S X B	97	91	20 a	48 b	65 b	140 a	96	3.6 a	4.7 a	57
	1199	B X B	96	95	22 a	52 a	73 a	150 a	89	3.5 a	4.5 a	60
											7 year	
80B	1193	S X S	94	94	10 c	34 b				1.9 b	1.2 b	92
	1194	S(self)	92	92	8 d	28 c				1.6 c	.9 c	86
	1195	S X B	100	96	13 b	46 a				2.4 a	2.4 a	96
	1196	S X S	100	100	9 d	36 b				2.0 b	1.5 b	92
	1197	S(self)	100	100	8 d	29 c				1.3 c	.7 c	92
	1198	S X B	98	92	14 b	45 a				2.5 a	2.4 a	96
	1199	B X B	98	98	14 b	48 a				2.4 a	2.4 a	100
	1123	B(open)			16 a	50 a				2.4 a	2.0 a	100
											11 year	
80C	1193	S X S								3.3 c	3.4 b	100
	1194	S(self)								2.9 d	2.6 c	100
	1195	S X B								4.8 a	5.8 a	96
	1196	S x S								3.3 c	2.3 b	100
	1197	S(self)								2.3 d	2.4 c	100
	1198	S X B								4.1 b	5.1 a	100
	5638	B X S								4.6 a	5.4 a	93
	5639	B X S								4.7 a	5.6 a	100
	Pet I	B X B								3.8 a	4.0 b	100
	Pet II	B X B								4.2 b	4.1 b	96
	Kap I	B X B								3.4 c	3.6 b	100
	Kap II	B X B								3.3 c	3.3 bc	100

[1] Data followed by the different letters fall into different, discrete, non-overlapping groups (Gates and Biblo 1978).

Table 4. Germination, growth, and survival of black (B), Serbian (S) and hybrid spruces

Experiment	Population	Species or hybrid	Seed No.	Seed Germinated %	Greenhouse Height cm	Nursery Height cm	Plantations Height cm	Plantations Survival %	Plantations Height cm	Plantations Survival %
82					10-weeks	2-years	10-years			
	SP58	B X S	49	90	3.3 a	43 a	300 b	85		
	SP63	B X S	3	33	3.3 a	-	-	-		
	SP67	B X S	4	75	3.4 a	49 a	370 a	100		
	MS46	(open)	50	89	2.9 b	30 b	340 a	70		
100 A,B,C,					6-months		2-years		6-years	
& 134	1548	B(open)	200	77	23 c		-	-	138 a	89
	1610	B X S	200	82	26 b		81 a	88	162 a	73
	1615	B X S	200	95	27 b		66 b	94	143 a	75
	1619	B X S	200	51	31 a		78 a	94	154 a	83
	1625	S(open)	200	93	19 d		45 c	83	107 b	85
	1626	S(open)	200	44	21 c		48 c	85	95 b	63
	2539	S(open)		-			75 a	94	-	-
105 A,B							6-years			
	1322	B(open)	50	98			166 a	85		
	1625	S(open)	50	71			96 b	91		
	1626	S(open)	50	49			103 b	98		
	2191	B X S	37	100			177 a	81		

Table 4. Cont'd

Experiment	Population	Species or hybrid	Seed No.	Seed Germinated %	Greenhouse Height cm	Nursery Height cm	Plantations Height cm	Plantations Survival %	Plantations Height cm	Plantations Survival %
118 A,B					5-months		5-years			
	1322	B(open)	200	98	10.0 a	40 b	135 a	97		
	1625	S(open)	200	39	8.5 b	29 c	84 b	75		
	2215	B X S	157	90	10.3 a	48 a	121 a	88		
	2216	B X S	8	75	9.4[1]	38 b	98 b	100		
123					6-months	2-years	3-years			
	1322	B(open)	200	93	18 a	67 a	89 a	100		
	1625	S(open)	200	48	11 b	25 b	38 b	71		
	2403	B X S	2	100	-					
	2404	B X S	1	0	-					
	2405	B X S	9	56	17 a	54 a	74 a	100		
	2406	B X S	17	82	20 a	63 a	101 a	100		
	2407	B X S	29	69	17 a	71 a	90 a	75		
	2408	B X S	69	99	17 a	56 a	80 a	83		
138,141					6-months	2-years				
	2709	B X B	150	93	28 a	56 a, 56 b				
	2706	B X S	150	93	17 c	43 c, 51 b				
	2712	B X B	150	98	25 b	51 b, 55 b				
	2711	B X S	150	94	23 b	42 c, 54 b				
	2717	B X B	150	99	29 a	57 a, 60 a				
	2714	B X S	108	96	25 b	45 c, 59 a				
	2744	S(open)	150	74	16 c	21 d -				

[1] Only 4 seedlings, not included in analysis.

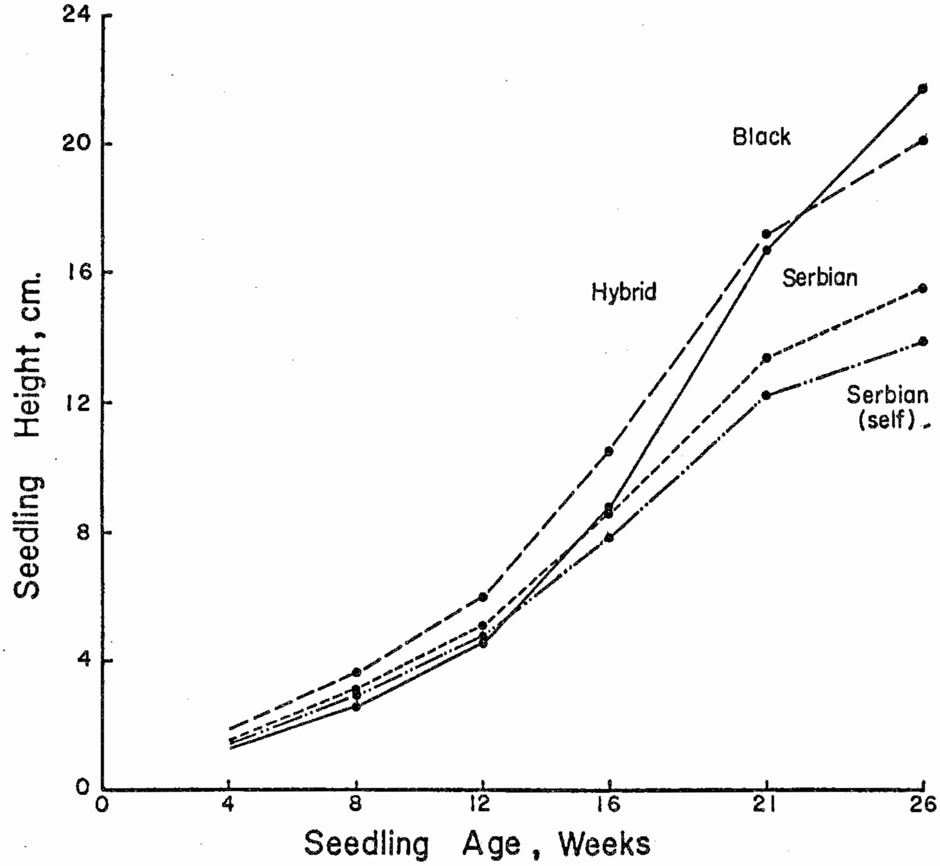

Fig. 2. Seedling height (cm) of black, Serbian, and Serbian X black seedlings at ages up to 26 weeks in a greenhouse.

In the greenhouse tests, the hybrids were consistently taller than the crossed Serbians which in turn were taller than the selfed Serbians. Black spruce, on the other hand, improved its relative position throughout the growth period (Fig. 2). At 8 weeks from germination, the black spruce seedlings were the smallest, by 16 weeks they were as tall as the Serbian spruce, at 21 weeks they were almost as tall as the hybrids and at the end of the greenhouse test (26 weeks) they had surpassed (not significantly) the hybrids.

In all nursery and field tests, selfed Serbian spruce was inferior to crossed Serbian spruce which in turn was inferior to the black and hybrid spruces. Black spruce retained a small height advantage over the hybrids throughout the nursery growth period (80A, B) and into the field plantings. After 4 years in the field, the black spruce was no longer significantly taller than the hybrids. At the end of seven (Nova Scotia 80B) and eight (AFES 80A) growing seasons, the hybrids were equal to or exceeded black spruce in both height and diameter.

The comparison between black spruce and the hybrids in experiment 80C (PNFI) is somewhat tenuous. The black spruce "controls" were raised under different conditions than the control pollinated materials. Although all seedlings were comparable in size when field planted,[2] it is not possible to separate non-genetic preconditioning effects from differences arising from genetic

[2] Personal communication, E.K. Morgenstern PNFI. Correspondence on File MFRC 6-12-1978.

causes. The Petawawa "controls" were sown in a greenhouse in February 1968 and planted into the test in the spring 1969. The Kapuskasing "controls" obtained from a provincial nursery at Spruce Falls, Ontario, as 2-2 stock were planted in the spring 1969. The Petawawa and Kapuskasing "controls" were two years younger and two years older, respectively, than the hybrid families. If one assumes the preconditioning effects to be small, the hybrids are equal to or better than the best black spruce in height, and considerably better than black spruce in diameter growth. The four hybrid families averaged 14% taller and 36% greater in diameter than the best black spruce (Pet. I and II). A less tenuous comparison of black and hybrid spruces can be made in height/diameter ratios. The hybrids are relatively larger in diameter (ht/diam = 83) than either Serbian spruce (100) or black spruce (98).

Non-genetic effects resulting from the direction of the cross, i.e., Serbian or black used as female parents, were not evident after 11 growing seasons despite the fact that the Serbian X black hybrids started from considerably larger seeds than the black X Serbian hybrids.

Survival of all populations in experiment 80A was high through the second year from field planting, but between the second and fourth year there was a marked increase in mortality in all the Serbian spruce populations. Between the fourth and eighth year, mortality was a serious problem in the black and especially in the hybrid spruce populations. The cause of mortality is not known although many of the trees showed evidence of damage by the shoestring fungus, *Armillaria mellea* (Vahl.) Quel. At 8 years from field planting, survival of the hybrids was 54% compared to 60% for black spruce, 20% for crossed Serbian spruce and only 10% for "selfed" Serbian spruce. The difference in survival between black spruce and the hybrids was not significant.

Survival of all families in experiments 80B and 80C was high after 7 and 11 years, respectively.

Experiment 82 Germination of the hybrid seeds was high (86%) and essentially the same as that of the black spruce controls. Abnormal germinants were not recorded. At 10 weeks of age in the greenhouse and after 2 growing seasons in the nursery, the hybrids were taller than black spruce. Only two hybrid families were field planted at AFES and one of these consisted of only three seedlings. After 10 growing seasons in the field, one hybrid family (SP67) was taller and the other (SP58) was shorter than black spruce. In general, the hybrids survived better than black spruce.

Experiments 100 and 134 Except for one family (1619) of which only 51% of the seeds germinated, the hybrid seeds germinated well. No abnormal germinants were recorded. After 6 months in the greenhouse (Exp. 100), the hybrids were taller than the black and Serbian spruce controls. After 2 years in the field, the mean height of the hybrids and black spruce was the same and exceeded that of the Serbian spruce. At the end of the sixth growing season in the field (Exp. 100A, B, and C) seedlings of the three hybrid families were taller (not significant) than black spruce on all three test sites. Serbian spruce was clearly inferior to black and hybrid spruces. The hybrids were 27% taller than the mean parent height and 11% taller than the tallest parent (black spruce). After 6 years in the field, survival of black spruce (89%) exceeded that of the hybrids (77%) and Serbian spruces (74%).

Experiment 105 Germination of seeds of black spruce and the hybrids was high and no abnormal germinants were recorded. Growth of both species and hybrids was better on the fresh site (site 4) than on the drier site (site 7), however, survival was better on the drier site. In both test plantations, after 6 years the hybrids were taller (not significant) than black spruce which in turn was considerably taller than Serbian spruce. The hybrids were 33% taller than the mid-parent height and 7% taller than the best parent (black spruce). Survival of the hybrids (81%) was somewhat poorer than that of black spruce (85%) and Serbian spruce (95%).

Experiment 118 Germination of black and hybrid spruce seed was high and no abnormal germinants were observed. After 5 months in the greenhouse, height of the black spruce and the hybrids was essentially the same and exceeded that of Serbian spruce. At the end of the second growing season in the nursery, the hybrids were as tall or taller than black spruce but after 5 years in the field the black spruce was taller (not significantly) than the hybrids and had somewhat better survival. Both the hybrids and black spruce were taller and survived better than Serbian spruce. The mean height of the two hybrid families was 1% greater than the mid-parent height but 14% less than the height of the best parent, black spruce.

Experiment 123 Average germination of the hybrid seeds was 86% compared to 93% for black spruce and only 48% for Serbian spruce. Six percent of the hybrid seeds germinated abnormally, i.e. reverse germination or double embryos. After 6 months in the greenhouse, 2 years in the nursery, and 3 years in the field, the hybrids and black spruce were not significantly different in height and both were clearly superior to Serbian spruce. The mean height of the hybrids exceeded the mid-parent height by 22% but was 3% lower than that of black spruce. Overall survival of the hybrids (85%) was lower than than that of black spruce (100%) but higher than Serbian spruce (71%).

Experiments 138 and 141 Germination of the hybrid and black spruce seeds was uniformly high. However, a higher proportion of the hybrid seed germinated abnormally (4% reverse embryos) compared to seeds of Serbian spruce (1.5%) or black spruce (0%). After 6 months in the greenhouse and 2 years in the nursery, the black spruce families were slightly but consistently taller than the hybrid families derived from the same female parents. Serbian spruce was consistently shorter than both the black and hybrid spruces.

Hybrid Verification

The high yields of full seeds from controlled crosses of Serbian and black spruce, the high rate of repeatable successful crosses and the failure of unpollinated controls to produce full seeds combine to provide strong evidence that the putitive hybrids are in fact authentic. The relatively high frequency of abnormal germinants, i.e., reverse germination or multiple germinants, in the hybrid families (5-6%) compared to the low frequency among parental populations (<1%) is also evidence that the crosses were successful. Additional evidence of hybrid authenticity can be derived from morphological comparison of putitive hybrids with the parental species. Intermediacy in most morphological characteristics is considered to be strong evidence of hybridity.

Measurements and observations made in conjunction with experiments 80, 82, and 118 (Table 5) indicate that hybrid families can be distinguished

Table 5. Hypocotyl color and number of cotyledons of black (B), Serbian (S), and hybrid spruces

Experiment number	Population number	Species or hybrid	Hypocotyl color[1]	Cotyledon number
80 A,B	1193	S X S	0.03	5.7
	1195	S X B	1.57	5.4
	1196	S X S	0.03	5.8
	1198	S X B	1.77	5.3
	1199	B (open)	3.58	4.3
82	SP 58	B X S	3.8	4.8
	SP 63	B X S	4.0[2]	4.0[2]
	SP 67	B X S	4.0	5.0
	$\bar{x}$ 13 trees	B X B	3.6	4.2
118	2215	B X S		5.8
	2216	B X S		6.3
	1322	B (open)		5.2
	1625	S (open)		5.9
	1626	S (open)		5.6

[1] 0 = no red pigment; 4 = lower half of hypocotyl red, see text.

[2] Mean of only 3 seedlings.

Table 6. Mean ($\bar{x}$) standard deviation (SD) and Character State Distance (CDS) of black and Serbian spruce for each of 16 characters examined

Character	Black spruce		Serbian spruce		CSD
	$\bar{x}$	SD	$\bar{x}$	SD	
Needle					
Thickness, mm	1.84	0.23	1.71	0.22	-1.29
Width, mm	2.14	0.39	3.16	0.45	1.16
Width:thickness	0.85	0.15	0.54	0.05	1.37
Rows stomates, abaxial	7.61	1.66	10.3	1.3	0.91
Rows stomates, adaxial	3.65	1.34	0.0	0.0	1.63
Color:chroma	5.70	0.71	5.86	0.50	-5.32
value	4.60	0.49	4.93	0.25	-0.21
hue	5.07	1.42	4.18	1.19	-0.94
Cone					
Scale margin shape	2.76	0.83	0.75	0.26	1.46
Scale length, cm	1.14	0.12	1.36	0.35	-0.17
Scale width, cm	1.38	0.12	1.13	0.30	0.30
Scale length:width	0.82	0.07	1.20	1.10	-1.09
Tip shape, degrees	76.7	4.9	57.3	4.8	1.49
Length, cm	2.60	0.27	4.51	0.56	1.56
Width, cm	2.23	0.31	2.69	0.41	0.47
Length:width	1.21	0.28	1.62	0.16	1.12

from parental families at an early stage of development. Hypocotyl color provided a good basis for separating hybrids from parents in experiment 80 where Serbian spruce had virtually no red pigments, black spruce was highly pigmented and the hybrids were intermediate. However, in experiment 82 where black spruce was the female parent, both hybrids and black spruce had highly pigmented hypocotyls. Cotyledon number (experiments 80, 82, and 118) also proved useful in distinguishing hybrid families. In experiment 80, mean number of cotyledons was clearly intermediate between the parental species. In experiment 82, the hybrids had more cotyledons than black spruce (female parent) and in experiment 118, the number of cotyledons on the hybrids approached that of the male parent (Serbian spruce). Although it is usually possible to distinguish hybrid and parental families on this basis, within family variation in respect to these traits precludes positive identification of individual seedlings.

The general overall appearance of hybrid seedlings is intermediate to and distinguishable from parental species seedlings at 6 months to 3 years of age. The basis for distinguishing hybrids and parents at this stage was not studied; however, when both parental species and the hybrid are present in the greenhouse and nursery the hybrids can be identified accurately and with reasonable ease. Older seedlings, e.g., 5 to 11 years, are intermediate between and readily distinguishable from parental species on the basis of overall appearance.

The means, standard deviations, and CSD for each of the 16 characters studied are listed in Table 6. Mean values for the hybrid families for all characters having a positive CSD, as well as the Affinity Indices, are listed in Table 7. Affinity Index values (Harrison and Valentine 1972) can range from 0 to 2 with 0 indicating that the hybrid's character state falls exactly between that of the parental species. The Affinity Indices established for the 14 hybrid families range from 0.02 to 0.75 and clearly support the supposition that they are hybrids.

Interestingly, the Affinity Indices for the younger families (5 to 6 years) tend toward black spruce whereas those of the older families (8 to 13 years) tend toward Serbian spruce. This difference relates to changes in needle conformation of the hybrids. The juvenile needles are square and similar to black spruce but gradually become flattened and similar to Serbian spruce as the tree matures.

DISCUSSION

Growth

Black spruce exhibits better height growth, at least over the first 10-15 years from planting, than any other spruce species tested in the Maritimes Region of Canada (Fowler and Coles 1979). In all tests reported here, the height of black spruce exceeded that of Serbian spruce.

Hybrid vigor, or heterosis, in the genetic or statistical sense is defined as the significantly better performance of hybrid offspring with respect to the average performance of the two parents. More commonly, hybrid vigor is used to describe hybrids that outperform both parents and in this sense is synonymous with "luxuriance" as defined by Dobzhansky (1964).

In the genetic or statistical sense, hybrids of Serbian and black spruce are clearly heterotic. The mean height of only one family (MS 2216 represented by only three seedlings) failed to exceed the mid-parent value in height growth. On

Table 7. Mean and Affinity Index for hybrid families for each character with positive CDS

Character	Population number (age-years)													
	SP58 (13)	SP67 (13)	1195 (13)	1198 (13)	1610 (9)	1615 (9)	1619 (9)	2191 (8)	2215 (6)	2216 (6)	2405 (5)	2406 (5)	2407 (5)	2408 (5)
Needle														
Width, mm	3.07	2.66	2.97	3.01	2.78	2.79	2.75	2.40	2.28	2.30	2.36	2.41	2.24	2.33
Width:thickness, mm	0.64	0.70	0.65	0.67	0.67	0.71	0.70	0.76	0.75	0.84	0.69	0.83	0.82	0.75
Rows stomates, abaxial	10.50	9.16	11.0	11.50	8.90	10.40	9.60	8.20	7.90	7.50	9.00	7.30	6.90	8.10
Rows stomates, adaxial	2.70	1.67	2.20	3.28	1.78	0.70	1.75	1.28	1.71	2.00	1.75	3.12	1.10	1.12
Cone														
Scale margin shape	1.56	1.00	1.83	1.40										
Scale length:width	1.28	1.23	1.31	1.34										
Tip shape	60.90	62.00	62.00	65.10										
Length, cm	3.39	3.35	3.60	3.41										
Width, cm	2.40	2.70	2.51	2.36										
Length:width	1.41	1.24	1.44	1.30										
Affinity Index*	0.107	0.386	0.595	0.020	0.113	0.064	0.157	0.444	0.399	0.429	0.092	0.747	0.438	0.278

* Underlined affinity indices indicate a trend toward black spruce, others indicate a trend toward Serbian spruce.

average, the hybrid, Serbian X black and reciprocal were 20% and 25%, respectively, taller than the mean of the parent species. Many of the hybrid families also exhibited hybrid vigor or "luxuriance" in that they were taller than the tallest parent, i.e., black spruce. However, the expression of hybrid vigor is highly variable and clearly related to age. This relationship is presented in Fig. 3. The Serbian X black spruce hybrids were consistently taller than black spruce for the first 4 to 5 months in the greenhouse. This early superiority undoubtedly is related to the larger seeds of the hybrid. After 5-6 months in the greenhouse the hybrids and black spruce are of about equal height (Fig. 2). Hybrids of the reciprocal cross that have no seed-size advantage are generally somewhat shorter (although not significantly) than black spruce throughout the period in the greenhouse. Regardless of the direction of the cross, black spruce is, on average, slightly taller than the hybrid during the nursery stage and for the first 4 or 5 years after field planting. Of the 16 comparisons that can be made between height of black and hybrid spruces after 2 to 5 years in the field, black spruce is taller in 12 and averages 7.1% taller than the hybrids.

The relative height growth of the hybrid increases after about 5 years from field planting. In all but one of the 14 comparisons that can be made between black and hybrid spruces that are 6 to 11 years from planting, the hybrids are taller ($\bar{x}$ = 7.4%) than black spruce.

Diameter breast height was measured in only one experiment (exp. 80) which was planted at different times and in three locations. Superiority of the hybrids is evident in only the oldest materials (11 years from planting) where the hybrids have an average of 35% more diameter than the best parent, black spruce.

These results are in general agreement with those of Gordon (1976) who reported no evidence of hybrid vigor in young hybrids of Serbian and black spruce and of Jeffers (1971) who reported 8-year-old hybrids to be heterotic. Rauter's (1971) report that young seedlings of Serbian X black and reciprocal are heterotic is not surprising considering the variability in the early performance of the hybrids demonstrated in this study. These hybrids continue to be heterotic after 12 field growing seasons (Rauter 1979a).

Survival

Survival of Serbian, black, and hybrid spruces in the greenhouse and nursery is uniformly high. Survival in the field is much more variable. Average survival of the hybrid families for all experiments and all sites is 87.8% compared to 88.2% for black spruce and 73.7% for Serbian spruce. Except for experiment 80A where there was a marked increase in mortality between year 4 and 8 from planting, most mortality occurred during the year of planting.

Experiment 80A is growing on a moderately fertile, fresh, mixedwood site at AFES. Seventy-three percent of the Serbian spruce, 43% of the hybrids, and 22% of the black spruce died between the fourth and the eighth growing season. Many of these trees showed evidence of damage by *Armillaria mellea* (Vahl.) Quel. Streets (1962) considers Serbian spruce planted in Great Britain to be highly susceptible to this fungus. It appears that the Serbian X black spruce hybrids are intermediate between the parental species in respect to susceptibility to *Armillaria mellea*.

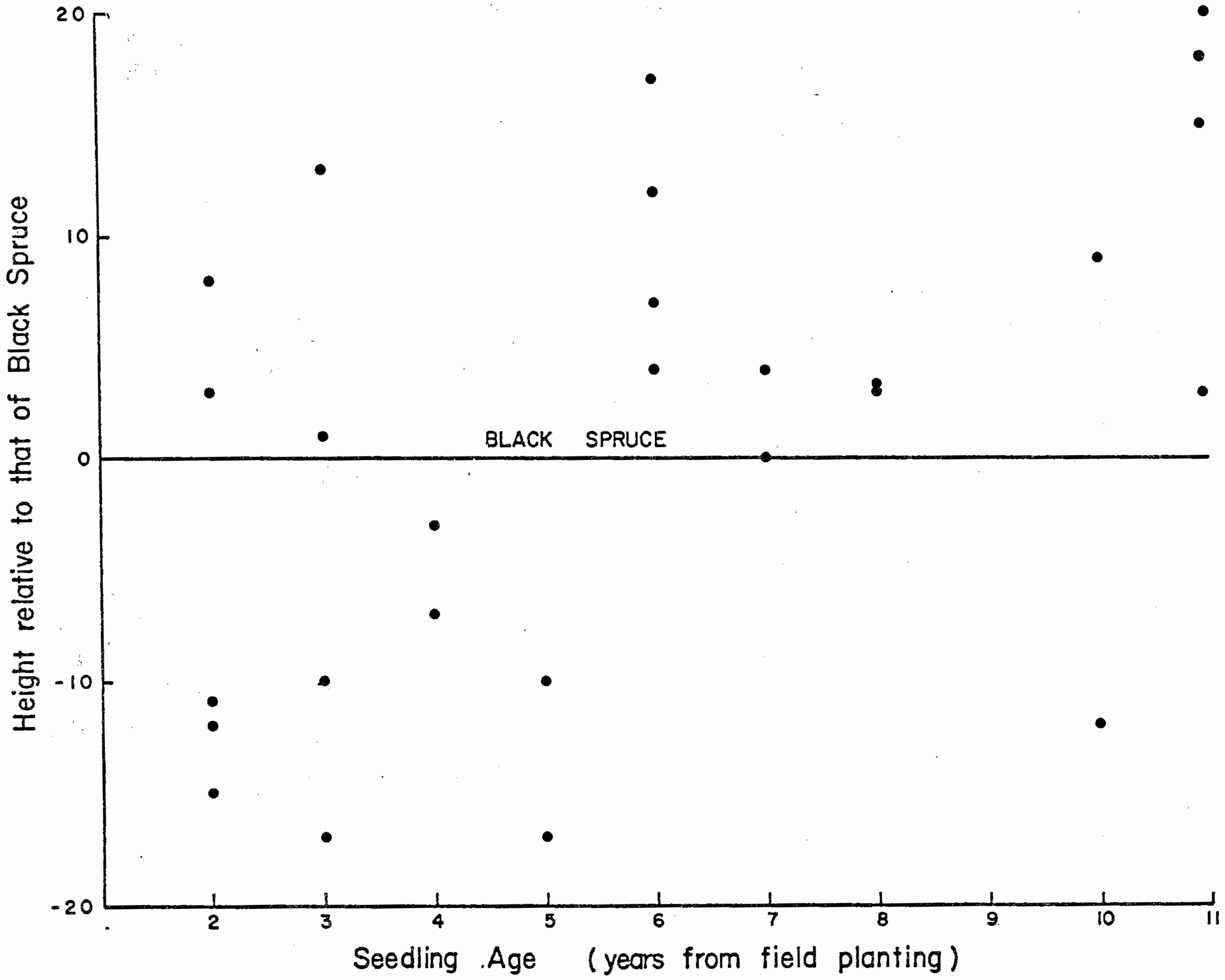

Fig. 3. Height of hybrids of Serbian and black spruce relative to the height of the tallest parent, black spruce, at ages 2 to 11 years from field planting.

Species crossability

Twenty of the 23 attempts to cross black and Serbian spruce produced full seeds (Table 2). Two of the three unsuccessful attempts were made in 1972 with 1-year-old pollen which was known to be of poor quality. It is evident that most black spruce and Serbian spruce trees can be crossed, however, the success of the cross differs markedly between individual trees.

Truly meaningful estimates of species crossability can be made only if information from both intra-specific and interspecific crosses is fully comparable. In much of the work reported here, one of the pollens, most usually that of Serbian spruce, was provided by cooperators and, by necessity, was stored for 1 year. The control pollen (intra-specific) was in most cases fresh. Storage of pollen per se does not result in reduced seed set (Livingston and Ching 1967) however, considering the differences in handling, transportation, and storage some reduction in pollen viability and, in turn, reduced seed set must be expected.

Despite the constraint of not having completely comparable data from intraspecific and interspecific pollinations, an attempt has been made to estimate crossability using the formula:

$$\text{Crossability (\%)} = \frac{\text{No. full seed/cone interspecific}}{\text{No. full seed/cone intraspecific}} \times 100$$

The results are summarized in Table 2. The individual estimates of crossability are highly variable both for crosses attempted in the same year and for crosses made in different years. The low estimates of crossability based on the 1972 pollinations undoubtedly result from the low viability of the Serbian spruce pollen.

Estimates of crossability for the two Serbian X black crosses are 44% and 79% ($\bar{x}$ = 61.5%) whereas estimates for the 18 reciprocal crosses range from 0 to 200% ($\bar{x}$ = 23.1%). If however, the 1972, pollination data are omitted, the estimates of crossability for the black X Serbian cross averages 40.3% and the overall crossability of black and Serbian spruce is 43.9%.

In those experiments where a direct comparison can be made between parental species and hybrids (i.e., they have the same female parent), germination of the hybrid seeds averaged 1.7% less than that of the parent species. In addition, in those experiments where abnormal germinants were recorded, 4.0% of the hybrid seeds germinated abnormally compared to only 0.3% for the parental species. If reduced germination and increased frequency of abnormal germinants are taken into account the overall estimate of crossability of black and Serbian spruce is reduced to 41.3%. Gordon's (1976) and Nienstaedt's (1977) estimates of crossability of black and Serbian spruces, although somewhat higher, fall well within the range of crossabilities reported here.

In these experiments, and in experiments reported by Gordon (1976) and Nienstaedt (1977) there is evidence of reciprocal differences in species crossability. In general, the crossability estimates are higher when Serbian spruce is used as female parent. There is also a biological advantage in using Serbian spruce as the female parent in that the Serbian cones produce more and larger seed and open more readily than those of black spruce.

Phylogeny

Species crossability has been used as a means of determining phylogenetic relationships within conferous genera (Wright 1955, Critchfield

1973). It is assumed that the more closely related two species are, the more readily they can be crossed. It is also assumed, that given enough time, species gradually differentiate genetically, and that specific barriers to crossing such as have been demonstrated for many angiosperms (Stebbins 1974) are not important in conifers (Critchfield 1973).

Black spruce and red spruce (*Picea rubens* Sarg.) are generally considered to be closely related (Wright 1955, Morgenstern and Farrar 1964, Morgenstern and Fowler 1969, Manley 1975, and Gordon 1976). Based on results from controlled pollinations of red and black spruce, Gordon (1976, 1977) reports mean crossability of these species to range from 0.7% to 3.2%. These estimates are considerably lower than those reported by Fowler et al. (1970, 1975) and Manley (1975). However, the general concensus is that Serbian spruce crosses more readily with black and red spruce than black and red cross with each other. It follows, if we accept crossability as a measure of phylogenetic relationships, that black spruce is more closely related to Serbian spruce than it is to red spruce.

Because almost all coniferous genera are represented around the edge of the Pacific Basin, Li (1953) suggested that this area was the most likely place of origin for the conifers. Wright (1955) further suggested eastern Asia as the most likely place of origin for the spruces because of the large assortment of species and the presence of *P. koyamai* Shirasawa which he considers to be a primitive species. Neither of these suggestions are supported by reliable fossil evidence.

Accepting eastern Asia as the place of origin, most authorities consider that the spruces reached North America via a land bridge connecting Siberia and Alaska either in a single migration (Hills and Ogilvie 1970, Nienstaedt and Tiech 1971) or in two or more independent migrations (Wright 1955, Gordon 1976). The close relationship of the northwestern American "white" spruces (*P. sitchensis* (Bong.) Carr, *P. engelmannii* (Parry) Engelm and *P. glauca* (Moench Voss) to each other and to eastern Asiatic *P. jezoensis* (Sieb. et Zucc.) (Wright 1955, Roche and Fowler 1975) supports this conclusion. The assumption that black and red spruce reached North America by the same route is more tenuous.

The fossil record indicates that intergeneric divergence in the Pinaceae took place some 135 million years ago during the late Jurassic or early Cretaceous period (Florin 1963). Comparative immunological studies (Prager et al. 1976) suggest that *Picea* was one of the first genera to emerge.

An eastern North American element was comparatively abundant in the flora of Europe during the Miocene and Pliocene (Szafer 1946). Well preserved fossils (cones, needles, and twigs) of a spruce, identified as *Picea rubra* Link. are listed among Pliocene fossils of Kroscienko, Poland. Szafer (1946) considers *P. rubra* to be essentially the same as contemporary red spruce. Other spruce fossils from the same location are identified as *P. excelsa* syn. *P. abies*, *P. polita*, *P.* cf. *Glehnii* and *P. omoricoides*. Despite the similarities between the Miocene-Pliocene floras of Europe and contemporary North American flora, Szafer (1946) did not consider the possibility of a land connection between Europe and eastern North America as a realistic explanation.

More recently, the general acceptance of the continental drift hypothesis and of a connection between Europe and North America lasting at least to the beginning of the

Triassic, (Dietz and Holden 1970, Smith and Briden 1977) makes such an explanation more feasible. It is quite conceivable that red spruce and possibly black spruce reached North America from the east. Another possibility, and one that fits the phylogenetic relationships based on crossability, is that Serbian spruce, or a close relative *(P. omoricoides?)* is ancestral to both red and black spruce. It is conceivable that red spruce reached North America, via Europe, during the Cretaceous or early Tertiary and became extinct in Europe sometime after the Pliocene (probably the Pleistocene). Black spruce, on the other hand, reached North America from the west via an Alaska-Siberia connection and contact between red and black spruce has occurred fairly recently as suggested by Manley (1975).

Suggestions for mass production of hybrids

Nienstaedt (1977) suggests that the hybrid between Serbian and black spruce can be mass produced sexually in seed orchards. The data on crossability presented in this paper and unpublished information on phenology of flowering of the two species, provide support for this suggestion. The data also indicate that there would be an advantage in using Serbian spruce as female parent in a seed orchard. Nienstaedt (1977) suggests two possible orchard designs for mass propagation of this hybrid:

> "(1) A simple two clone design -- in such an orchard, seed would be harvested on both the *P. omorika* and the *P. mariana* and, except for a small portion of selfed seed, would all be expected to be of hybrid origin.
> (2) Orchards of one *P. omorika* clone and several *P. mariana* clones -- hybrid seed would be harvested only on *P. omorika*."

Of the two designs I prefer the latter. Serbian spruce is highly self fertile and seedlings resulting from selfing, although inferior to hybrid spruce, do survive and grow reasonably well (Langner 1959 and Exp. 82). In a two-clone orchard I anticipate an unacceptably high proportion of selfed seed on the Serbian spruce.

A third possible orchard design, and one that could be used to advantage in the Maritimes Region of Canada, would be to utilize existing black spruce plantations of known good provenance as a pollen source and grafts of Serbian spruce as female parents. A practical program would be as follows:

1) Locate or plant, as part of the regular reforestation program, a 50-100 ha plantation of black spruce. The plantation should be located on a fertile upland site conducive to early and regular flowering.

2) When the plantation is 4-6 years old (1-1.5 m), field graft with scions of several (10-20) clones of Serbian spruce. To assure a minimum of intraspecific pollination on the Serbian spruce the grafts should be spaced 20 x 20 m. At this spacing 25 grafts could be established per hectare, so that a 50-ha orchard would contain 1250 grafts. According to Nienstaedt (1977) production would be about 117,000 full seeds annually at 6-8 years from grafting. Production would increase as the grafts mature.

Recent advances in vegetative propagation techniques provide an alternative means of mass producing spruce hybrids. Several schemes have been advocated for propagation of spruce by stem cuttings (Kleinschmit 1974, Nienstaedt 1977, Rauter 1979b). Methods developed and now employed on a large scale in Germany (Kleinschmit 1974) suggest that mass vegetative propagation is not only possible, but economically feasible. Using these methods, controlled pollinations are made between selected parents. The best seedlings are selected from among the resulting progenies at 2-4 years of age. Cuttings are struck and used to establish nursery trials. Cuttings are again struck from clones that pass the nursery test and used to establish additional tests or hedged for mass cutting production. Nienstaedt (1977) suggests that within 10 years annual cutting production for each selected clone could reach 20,000. Number of clones included in the program and time provide the major restraints on the numbers of plants produced.

CONCLUSIONS

Of the many spruce hybrids that have been produced and tested in eastern Canada, hybrids of Serbian and black spruce are among the most promising. The hybrids are clearly heterotic in the statistical sense (exceed the mid-parent mean) and in most trials, 5 years or older from field planting, the hybrids out-perform the best parent (black spruce) in height and diameter growth.

Mass production of the hybrids in seed orchards or by rooting of cuttings appears feasible.

Serbian and black spruce bear the closest relationship, demonstrated to date, between Old World and New World spruces. Based on crossability, black spruce is more closely related to Serbian spruce (mean crossability 44%) than it is to its closest North American relative, red spruce.

It is suggested that Serbian spruce is ancestral to black spruce and red spruce. Although the evidence is tenuous, it is also suggested that black spruce reached North America from the west via a Bering land connection, that red spruce reached North America from the east (prior to continent separation), and that red spruce is older than black spruce. It follows that contact between red and black spruce could be a relatively recent occurrence as suggested by Manley (1975).

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