VARIATION IN COTYLEDON NUMBER AND SEED WEIGHT IN WHITE SPRUCE IN ALBERTA

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ABSTRACT

The average cotyledon number for 7,909 seedlings of white spruce from 37 samples in Alberta was 5.6/seedling. Averages among samples ranged between 4.8 and 6.6. These 37 samples had a high proportion of seedlings with four cotyledons compared to samples from eastern Canada. Average seed weights per sample ranged between 1.26 and 3.24 mg/seed giving a common average 196,000 seeds/pound. Any two seed samples which differed in their average cotyledon numbers by more than 0.3 of one cotyledon were statistically different. Coefficients of variation in average cotyledon numbers per sample were the same for single trees and groups of trees indicating that the single trees in this study were fertilized by many pollen donors. The higher coefficients of variation in samples with low, rather than high, numbers of cotyledons suggest white spruce gene mixing with black rather than Engelmann spruce in Alberta. Average seed weights per sample in white spruce are useful for describing differences among forest regions while average cotyledon numbers are highly specific for each seed sample regardless of geographic origin. The relationship between seed weight and cotyledon number is offered as an added criterion for the distinction of particular populations of white spruce.

INTRODUCTION

Conifers produce seedlings with more than two cotyledons (Butts and Buchholz 1940). The number per seedling varies within a given species, but it has been used effectively to distinguish hybrids between species (Silen *et al.* 1965). The number of cotyledons per seedling has also been used to describe differences among subpopulations within a species (Klein 1958; Sorensen 1966; Thorbjornsen 1961). In addition, the measure of average seed weight has been used successfully as a criterion for species characterization (Simak 1967; U.S.D.A. 1948).

White spruce [*Picea glauca* (Moench) Voss] differs from *P. mariana* (Mill.) BSP. and *P. engelmannii* Parry in number of cotyledons (Brayshaw 1959; Franklin 1961) and in average seed weight (U.S.D.A. 1948). Where the geographic distribution of

white spruce overlaps that of both other species (Fig. 1), and where there are frequent hybrids between white and Engelmann spruce (Horton 1959) or potential natural hybrids between white and black spruce (Little and Pauley 1958), cotyledon number may have value as a criterion for describing interspecific spruce hybrids.

The main objective of this study was to determine if there are regional differences in average cotyledon number or seed weight in white spruce in Alberta and if such differences are associated with geographic proximity to the other spruces. A complimentary objective was to determine if cotyledon number is correlated with seed weight in the same species.

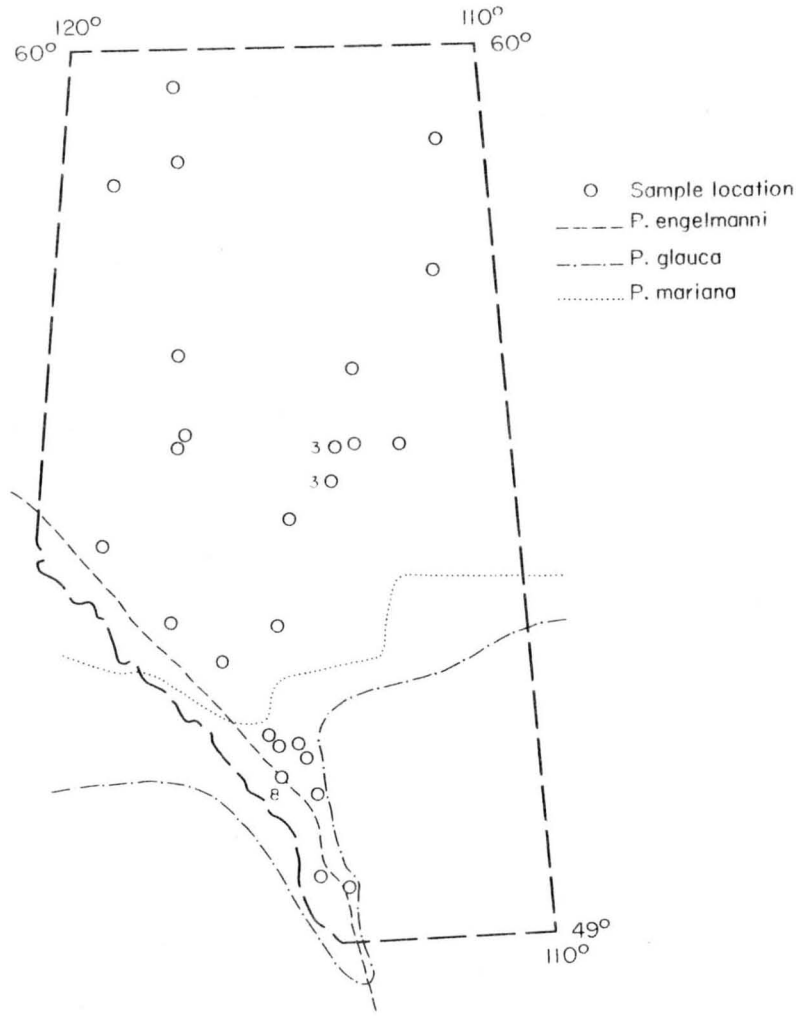


Figure 1. Location of 37 samples of white spruce seed, the eastern limits of P. engelmannii, *and the southern limits of* P. mariana *and* P. glauca.

MATERIALS AND METHODS

Forty different seed lots of white spruce from different geographic areas are described in Table 1. Twenty-three samples were collected between 1959 and 1967 from groups of wind pollinated trees in various parts of Alberta (Fig. 1)[1]. Most of these seeds were collected in late August or throughout September. In addition, 14 collections were made in late August and early September in 1964 and 1966 from single wind pollinated trees located southeast of Lesser Slave Lake and in southwestern Alberta near the Kananaskis Forest Experiment Station. These 37 samples cover the range of white spruce in Alberta. The remaining three samples were collected from single wind pollinated trees near Wasagaming, Man., in the middle of August in 1964.

Average seed weights, measured to the nearest tenth of a milligram, are shown for each seed lot in Table 2. At the time of weighing, moisture contents of air dry seed varied between 3% and 12% (Table 2). All samples contained less than 8% empty seeds (Table 2). Single seeds from individual trees were weighed separately for correlation tests between seed weight and cotyledon number.

The usual germination regime of 30-20C (day-night cycle) which is recommended for white spruce (U.S.D.A. 1948; Heit 1961) was not satisfactory because it facilitated fungal and bacterial growth which decreased total germination and killed many germinants before cotyledons could be counted. Surface sterilization with mercuric chloride (Chen and Jong 1965) was only partially effective in preventing losses from fungi and bacteria. Best results were obtained when unsterilized seeds were placed on moist blotters in petridishes in a germinator which 20-10C day-night temperatures. The seeds were exposed to 500 ft-c of fluorescent light during the 12-hour days.

Cotyledons were counted 10 to 20 days after germination or as soon as they emerged from the seed coats.

RESULTS

Cotyledon Number and Seed Weight Variations in White Spruce in Alberta

Most seedlings of white spruce had between four and seven cotyledons. Seedlings with three, eight, or nine cotyledons

[1]These seeds were kindly donated by the Alberta Forest Service in 1967.

Table 1. Geographic Locations of 40 Seed Samples of White Spruce from Alberta and Manitoba. The First Two Numbers in Each Seedlot Number Indicates the Year of Collection.

Source	Seedlot Number	Latitude °	Latitude '	Longitude °	Longitude '	Altitude ft asl.
Grande Prairie	64-111B	55	05N	117	10W	2,150
"	64-152	55	13	117	00	2,100
Rocky Mtn. House	59- 89	52	49	115	10	2,800
"	61-142	52	38	116	05	4,500
"	61-149	51	31	114	40	4,500
Crowsnest	60- 4	49	46	113	57	5,000
"	60-277	49	52	114	23	6,000
Whitecourt	59- 10	54	15	114	50	2,300
Edson	59- 60	53	58	118	37	3,000
"	60- 35	53	04	117	15	5,000
Lac La Biche	59- 19	58	45	111	00	800
"	59- 80	57	15	111	21	750
"	59- 82	55	05	112	26	1,900
Peace River	60- 27	58	25	118	45	800
"	59-143	59	37	117	12	800
"	59- 8	56	16	117	05	1,900
"	67- 53	58	45	117	10	1,500
Slave Forest	59- 44	56	00	113	18	1,900
"	64- 45	55	13	113	25	2,025
"	66-169	55	05	113	53	2,100
"	66-171	54	39	113	56	2,200
"	66-172	54	39	113	56	2,200
"	66-175	55	12	113	57	2,050
"	66-176	55	12	113	57	2,050
"	64-100	54	39	113	56	2,200
Bow Forest and Kananaskis	62- 59	51	20	114	45	4,900
"	64-281	51	35	115	12	6,000
"	64-133	51	42	115	18	5,550
"	64-283	50	51	114	33	4,600
"	66-991	51	05	115	02	4,500
"	66-992	51	05	115	02	4,500
"	66-993	51	05	115	02	4,500
"	66-994	51	05	115	02	4,500
"	66-995	51	05	115	02	4,500
"	66-996	51	05	115	02	4,500
"	66-997	51	05	115	02	4,500
"	66- 13	51	05	115	02	4,500
Approximate locations of three Manitoba samples:						
Wasagaming	64- 35	50	50	100	00	2,200
"	64-116BL	50	50	100	00	2,200
"	64-116BR	50	50	100	00	2,200

Table 2. Average Seed Weights and Cotyledon Numbers Per Seedling in 40 Seed Samples of White Spruce from Alberta and Manitoba. The First Two Numbers in Each Seedlot Number Indicates the Year of Collection.

Seedlot Number	Avg Weight mg	No.	Avg No. Cotyledons/ Seedling	Seedlings No.	Empty Seeds/ Sample %	Seed Moisture Content when Weighed %
Alberta in general except Kananaskis, Bow Forest, and Slave Forest						
64-111B	2.1	200	5.5	48	3	5
64-152	2.0	200	5.4	154	2	5
59- 89	2.3	200	5.9	156	2	4
61-142	2.4	200	5.7	172	0	4
61-149	2.4	200	5.4	139	8	6
60- 4	2.6	200	5.8	61	4	4
60-277	1.9	200	5.2	122	7	3
59- 10	2.2	200	5.8	188	0	4
59- 60	1.7	200	4.8	86	6	4
60- 35	2.5	200	5.6	150	6	4
59- 19	2.4	200	5.8	126	6	5
59- 80	2.2	200	5.7	162	4	6
59- 82	2.2	200	6.0	156	6	5
60- 27	2.3	200	5.9	166	3	4
59-143	2.0	200	5.5	182	1	5
59- 8	2.3	200	5.9	185	3	5
67- 53	2.2	200	5.8	129	2	4
Slave Forest						
59- 44	2.2	200	6.0	9	8	12
64- 45	1.8	14000	5.4	531	1	5
66-169	1.8	270	5.4	240	0	5
66-171	2.5	258	6.2	177	0	6
66-172	2.1	383	5.6	323	0	5
66-175	2.0	251	5.4	147	0	6
66-176	2.5	288	6.0	225	0	6
64-100	1.3	2844	4.8	254	0	6
Bow Forest and Kananaskis						
62- 59	2.2	200	5.6	172	4	4
64-281	2.0	200	5.5	117	7	6
64-133	2.2	14000	5.5	604	5	5
64-283	1.9	14000	5.6	400	3	4
66-991	3.1	256	5.8	234	0	5
66-992	3.0	268	5.9	227	0	5
66-993	3.2	250	6.1	245	0	5
66-994	2.9	501	6.1	481	0	4
66-995	2.4	569	5.5	521	0	5
66-996	3.2	248	6.6	194	0	5
66-997	2.5	286	5.3	251	0	5
66- 13	2.9	249	6.6	154	0	4
Manitoba						
64- 35	2.7	2665	6.6	1087	0	5
64-116BL	1.9	3083	6.0	276	0	5
64-116BR	2.1	2559	5.9	326	0	5

(Table 3) comprised only 1.3% of the total population of 7,909 individuals. The average cotyledon number per seedling for the 37 seed sources combined was 5.6. Average cotyledon numbers among the 37 seed samples varied from 4.8 to 6.6.

Table 3. Percentage of 7,909 White Spruce Seedlings in each of Seven Cotyledon Classes. Data Based on 37 Samples from Different Geographic Areas in Alberta.

Number of cotyledons per seedling	Per cent of seedlings
3	.1
4	6.4
5	34.3
6	47.5
7	10.5
8	1.1
9	.1
Average number of cotyledons/seedling	5.6

Seed weights in the 23 samples donated by the Alberta Forest Service averaged between 1.72 and 2.64 mg while average seed weights ranged between 1.26 and 3.24 mg for the 14 single trees. The number of sound seeds per pound could therefore range between 139,000 and 357,000 depending on parentage. The overall average, based on all 37 samples from Alberta, was 196,000 seeds per pound.

No linear correlations could be established within Alberta as a whole between latitude, longitude, or altitude of seed sample and either seed weight or cotyledon number. Three dimensional plots between seed weight or cotyledon number, altitude, and latitude or longitude did not reveal any obvious interaction contributions from these geographic variables.

The data on seed weight and cotyledon number were therefore grouped into seven broad forest regions to determine if seed weights or cotyledon numbers from any one region differed from those of any other. The following areas were compared: Kananaskis and Bow; Crowsnest; Rocky Mountain House, Whitecourt, and Edson; Slave Lake; Grande Prairie; Peace River; Lac La Biche. Analyses of variance showed that there were regional differences in seed weight but not in cotyledon numbers at the 90% level of confidence. The seeds from the Kananaskis and Bow forests were significantly heavier (95% level) than those from the Slave Lake Forest but no other differences were described by this sampling material. The 18 samples collected in close proximity to the natural distribution of Engelmann spruce, from sites averaging 4,160 feet above sea level, were

also heavier (99% level) than those collected from the rest of the province where the elevation averaged 1,815 feet. The 18 samples weighed on the average 2.51 mg per seed while those distant from the mountains averaged 2.13 mg per seed.

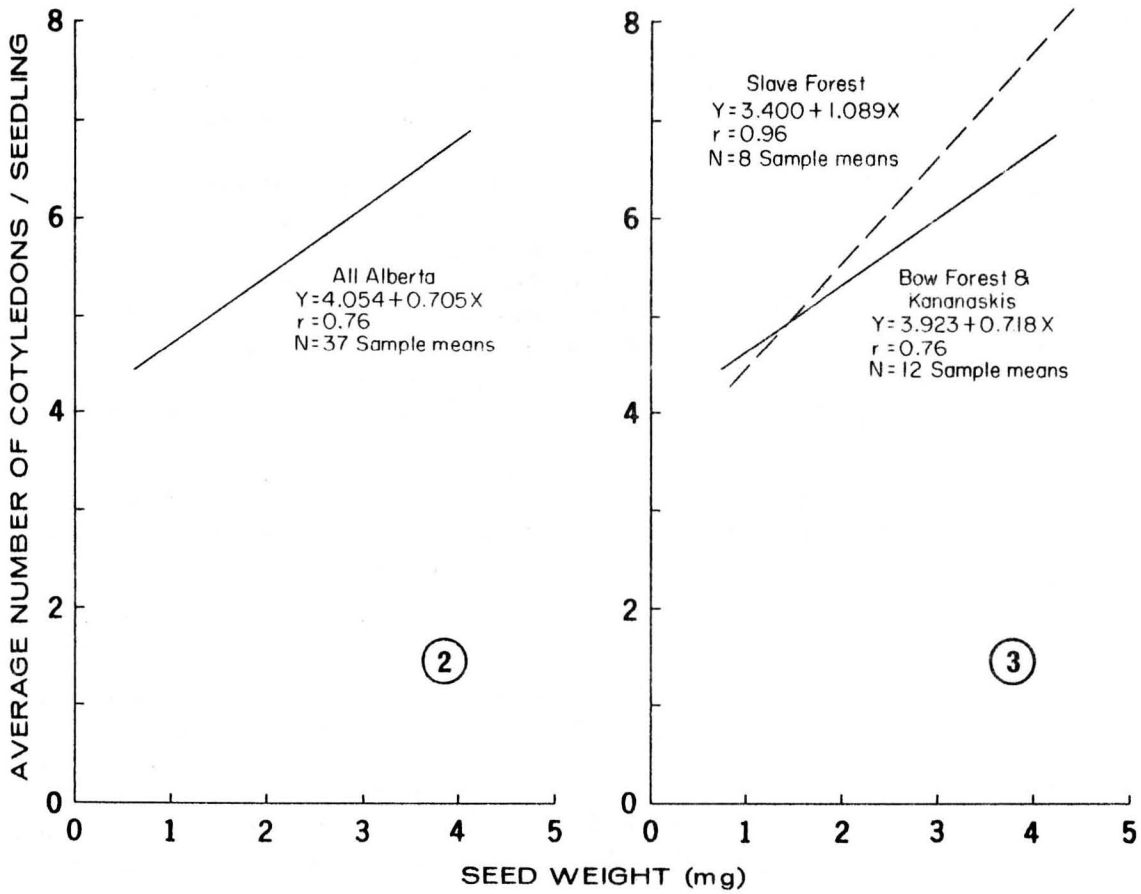
Cotyledon numbers per seedling were compared on a sample to sample basis disregarding geographic origins. The among-sample variation was highly significant (99.5% level) as expected because regional differences did not exist. Any two samples which differed in average number of cotyledons by more than 0.3 of one cotyledon were significantly different from each other at the 99% level of confidence (Duncan's Multiple Range Test). Only one sample was distinctly different from its closest neighbor when ranked by size. Sample 66-13 from the Kananaskis forest produced seedlings with more cotyledons than any other sample. Sample 64-100 from the Slave Lake forest and 59-60 from Edson produced seedlings with significantly fewer cotyledons (99% level) than all other samples.

A negative relationship exists at the 90% level of confidence between mean cotyledon numbers per sample and the coefficients of variation for the same means. As the mean number of cotyledons per sample decreased, the coefficients of variation increased significantly ($Y = 0.258 - 0.0231X$). There was no statistical difference between coefficients of variation in data from single trees (0.121) and groups of trees per sample (0.131) nor were there any differences in standard deviations (both with 0.70).

Correlation Between Cotyledon Number and Seed Weight in White Spruce in Alberta and Manitoba

On a province-wide basis, the 7,909 seedlings from the Alberta seeds demonstrated a positive correlation between seed weight and cotyledon number (Fig. 2). Because regional differences in seed weight did exist, regional differences in the relationship between cotyledon number and seed weight were sought. The total sample data for Alberta were scattered geographically and allowed only one regional comparison, namely that between the Slave Lake forest and the combined data from Kananaskis and Bow forests. Only the northern samples (Slave Lake forest) differed significantly in slope from the province-wide mean (95% level), but the samples from the Bow and Kananaskis forests were statistically different in slope from the ones collected from the Slave Lake forest (99.5% level). The samples from these two regions differed in average seed weights, as already indicated, but they had the same average cotyledon numbers.

The above relationships are based on pooled samples of seeds from several wind pollinated trees per location. In



Figures 2 and 3. Correlation between seed weight and cotyledon number in white spruce seed: 2, from 37 locations in Alberta; 3, from eight locations south east of Lesser Slave Lake and 12 locations in the Bow and Kananaskis forests.

contrast to this, individual seed weights plotted against the corresponding cotyledon number (Fig. 4) for individual wind pollinated trees illustrate different relationships. Samples from the three trees in Alberta and the three trees in Manitoba give very low correlations between seed weight and cotyledon number. The highest coefficient of determination (r^2) for all trees was 0.15. This is less than one third of the r^2 values obtained when pooled samples of seeds from several trees were tested (Figs. 2 and 3).

DISCUSSION AND CONCLUSIONS

The average cotyledon number per seedling in white spruce in Alberta is 5.6 (Table 3) which is similar to values reported for Ontario and the Maritimes (Ahlgren and Ahlgren 1958; Place 1955). However, the seeds from Alberta produced

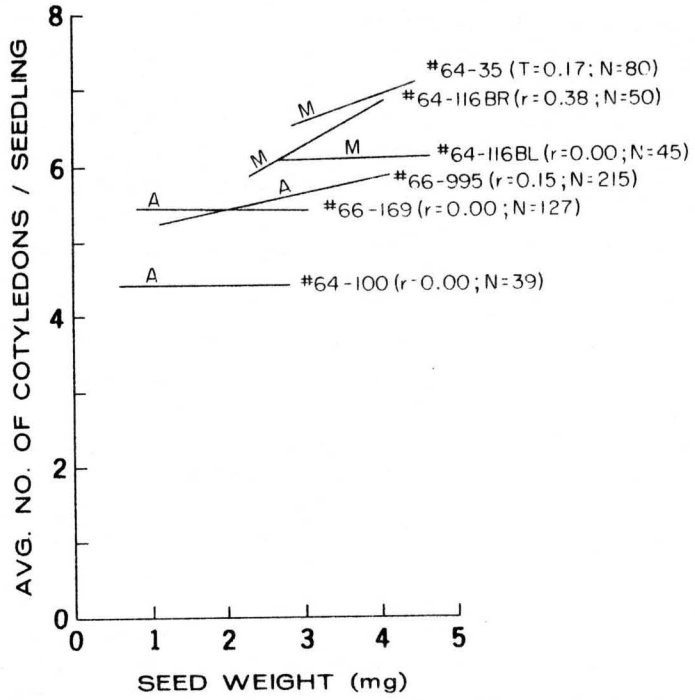


Figure 4. Correlation between seed weight and cotyledon number in white spruce seed from six wind pollinated trees; A - Alberta and M - Manitoba.

more seedlings with four cotyledons than those from eastern Canada. They also illustrated a greater range of cotyledons per seedling than reported hitherto.

The average number of seeds per pound, 196,000, representing samples from single trees and groups of trees in white spruce in Alberta, is intermediate between those quoted in the Woody-Plant Seed Manual (U.S.D.A. 1948) for two populations of white spruce. The range in averages among samples (139,000 to 357,000) is also intermediate between the two given in the Seed Manual.

Linear correlations between seed weight or cotyledon number per seedling in white spruce in Alberta and latitude, longitude, or altitude were not evident. Reasons for this are related to both environmental (Longley 1967; Morgenstern and Farrar 1964) and genetic influences. Three dimensional plots of seed weight or cotyledon number, altitude, and latitude or longitude of sample reveal only that the samples from the foothills in general and from the Kananaskis and Bow forests in particular are heavier than those from other parts of the Province. These heavier seeds may be a result of altitudinal influences on seed weight (Holzer 1966; Simak 1967) or a result of genetic mixing with Engelmann spruce which has a heavier seed than white spruce. They may also be heavier in comparison

to the samples from lower altitudes if the latter are influenced by gene mixing with black spruce which has a lighter seed than white spruce. Factors of site, year of collection, time of collection in fall, and duration of storage also influence seed weight (Baldwin 1942), but none of these are considered important in the comparison between samples from Kananaskis and Bow vs. Slave Lake forests. Most of these samples were collected from single trees in 1966 at the time of peak ripeness and were stored identically prior to testing.

Average cotyledon number per seedling varied among samples and not among forest regions. This parameter is therefore more highly specific of the seed sample than is seed weight. The observation that any two seed samples are significantly different if their average cotyledon numbers vary by more than 0.3 of one cotyledon and the wide range in average values (4.8 to 6.6) indicate how useful this criterion is for describing sample differences.

Cotyledon numbers vary as much in seed samples collected from groups of wind pollinated trees as in samples collected from individual wind pollinated trees. This supports the belief that single trees are fertilized by a large number of different pollen parents (Sarvas 1967).

The negative correlation between average cotyledon number and the coefficients of variation within samples furnish indirect evidence of crossing between white and black spruce. Genetically pure samples of either black or white spruce could be expected to contain less internal variation than genetically impure samples of either species. Because the sample data show greatest variation within samples of white spruce with low average cotyledon numbers, crossing with black rather than Engelmann spruce is suggested. The two samples with the smallest average cotyledon numbers were collected within the area of overlap between white and black spruce. The decreasing internal variation in samples with increasing average cotyledon numbers does not lend support to a species distinction between Engelmann and white spruce.

Most of the weight of a tree seed is composed of maternal tissues (Buchholz 1946) with only about 10% directly influenced by the pollen. On the other hand, the pollen contributes a theoretical 50% to the variation in cotyledon number in any seed. This is the reason why seed weight and cotyledon number are poorly correlated in seeds from individual wind pollinated white spruce trees. The highest coefficient of determination among the six trees studied was 0.15, which is similar to that found by Hadders (1967) for direct male contribution to seed weight. High correlations between seed weight and cotyledon number in single trees can only be obtained, therefore, if

seeds vary little in either seedcoat or endosperm weight within given seed weight classes.

Average seed weight has been shown to be a valid criterion for distinguishing regional populations of white spruce seed. Cotyledon number has been found to be highly specific to individual seed samples. It is suggested that a third criterion, useful in the identification of white spruce seed samples, is the relationship between seed weight and cotyledon number. However, this last criterion is most useful when samples from several trees rather than when single trees are compared.

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