ALBERTA SEED TESTING STANDARDS

Forestry Division
Alberta Sustainable Resource Development
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1.0 INTRODUCTION

Seed testing is done to assess seedlot attributes to determine overall quality and value for seedling production and storage. Seed testing standards provide set procedures for facilities to conduct tests in a uniform manner to ensure comparable results that are within acceptable ranges. The seed testing standards described below are closely aligned with International Seed Testing Association (ISTA) International Rules for Seed Testing Edition 2005 and Methods and Procedures for Testing Tree Seeds in Canada and follow these accepted procedures for sampling, moisture content determination, purity analysis, and seed weight determination. Germination testing standards have been modified based on protocols developed at the Petawawa National Forestry Institute.

These standards have been developed for parties that have a working knowledge of forest tree seed testing and are familiar with seed testing materials, instruments, and equipment. For a comprehensive description of general seed testing principles, procedures, equipment, and materials refer to International Seed Testing Association (ISTA) International Rules for Seed Testing or Methods and Procedures for Testing Tree Seeds in Canada.

2.0 Seed Testing Facilities Approval Process

Only seed test results conducted at facilities approved for seed testing by Alberta will be accepted. Interested parties can apply for Department approval by contacting the Provincial Seed Officer, Alberta Tree Improvement and Seed Centre. Applicants must submit written seed testing procedures used at their facilities, be subject to on-site audits by an Alberta representative, and conduct tests on seedlots supplied by Alberta and submit the results to Alberta for evaluation of accuracy of results. Approval will be granted when applicants can successfully demonstrate that tests can be conducted in accordance with these seed testing standards using appropriate equipment and materials and meet accuracy standards. Once approved, on-site facility audits will be conducted by Alberta at least once every three years or as determined necessary by Alberta. Facilities will be required to conduct tests on seedlots supplied by Alberta and submit the results for evaluation every three years or as determined necessary by Alberta.

Facilities testing Alberta seed prior to May 1, 2009 will have until January 01, 2010 to submit written seed testing procedures to Alberta and will be subject to periodic facility audits and accuracy testing upon receipt of Alberta's approval.

3.0 Reporting Results

Seed test data and results are provided to Alberta for review and entry onto the Seed Information Management System (SIMS) database. Data and results will be reviewed for completeness, accuracy and adherence to testing and submission requirements by the testing agency prior to submission to Alberta. Data must be submitted in the required format as outlined in Figs 2 and 3, or as otherwise approved with prior written consent of Alberta.

4.0 Sampling

The objectives of sampling are to obtain a sample that is suitable in size for testing and to obtain a sample that is representative of the lot being tested. Primary samples are small portions of seed taken at random from the seedlot. All primary samples taken from one seedlot are combined and mixed to form the composite sample. The composite sample is reduced to a smaller subsample called the submitted sample. The submitted sample is the portion of seed that is submitted to the laboratory for testing. The working sample is a subsample of the submitted sample and is the portion of seed on which a test is made.

The following sampling intensities shall be used.

1-4 containers	3 primary samples from each container
5-8 containers	2 primary samples from each container
9-15 containers	1 primary sample from each container
16-30 containers	15 primary samples from the lot taken from randomly selected containers with no more that one sample per container
31-59 containers	20 primary samples from the lot taken from randomly selected containers with no more that one sample per container

The primary samples are combined and thoroughly mixed to make a uniform composite sample. A random method must be used to reduce the composite sample to the required submitted sample size as indicated in Table 1.

After testing, remaining seed from submitted samples must be stored in a refrigerated area for one year from the date of receipt.

Table 1. Minimum weights for submitted samples and working samples for purity analysis by species.

Species	Submitted sample size for tests including moisture content (g)	Submitted sample size for tests not including moisture content (g)	Working sample size for purity analysis (g)
Abies balsamea	50	40	20
Abies lasiocarpa	50	50	25
Larix laricina	50	25	10
Larix sibirica	50	25	10
Picea engelmannii	50	16	8
Picea glauca	50	10	5
Picea mariana	50	6	3
Picea pungens	50	30	15
Pinus albicaulis	700	700	350
Pinus banksiana	50	25	9
Pinus contorta	50	25	9
Pinus flexilis	500	500	250
Pinus sylvestris	50	40	20
Populus spp.	5	5	2
Pseudotsuga menziesii	60	60	30
Betula papyrifera	10	10	3

5.0 Required Tests

5.1 Moisture Content Determination

The objective of a moisture content test is to determine the moisture content of a seed sample which is expressed as a percentage of the weight of the original sample. Moisture content testing must commence within 24 hours of receipt of the seed. Tests must be carried out in duplicate on two samples weighing 4 to 5 grams each, unless otherwise stated, that are independently drawn from the submitted sample. For small operational seedlots of less than 200 grams, reduced sample sizes and/or sample numbers are as follows:

50 to 200 grams of seed	use two 1-gram samples
30 to 50 grams of seed	use two 0.5-gram samples
10 to 30 grams of seed	use one 0.5-gram sample
<10 grams of seed	moisture content testing is not mandatory. Special emphasis should be given to proper handling procedures, drying and conditioning.

A sample can not be exposed to the ambient air for longer than two minutes from the time it is removed from the sample container until it has been placed in the drying container. Drying containers must be metal. If labeled, each container and its lid must have matching numbers. Containers and lids are dried for 1 hour at 130ºC and cooled in a desiccator before each use. An empty container and its lid must be weighed after the one hour pre-drying period and immediately prior to withdrawing a working sample. The sample is evenly distributed over the surface of the container. The container, lid, and the seed must be reweighed and placed into the oven. Samples must be dried at 103°C ± 2° for 17 hours ± 1 hour. At the end of the drying period, the lid must be placed on the container before the seed is cooled in a desiccator for at least 30 to 45 minutes. After cooling, the container, its lid and the seed are re-weighed. All weights are recorded in grams to three decimal places.

The following formula is used to calculate moisture content:

$$(M2 - M3) \quad x \quad \frac{100}{M2 - M1}$$

where M1 = weight of the container and its cover; M2 = weight of the container, cover, and contents before drying; and M3 = weight of container, cover and contents after drying. Moisture content must be expressed as a percentage of the weight of the original sample. The average of the two results is the percentage moisture content of the sample. If the results of the two samples differ by more than 0.3%, the test must be repeated.

5.2 Purity Analysis

The objective of purity analysis is to determine the percentage composition by weight of pure seeds, seeds of other species, and inert particles that make up the sample.

A working sample as prescribed in Table 1 must be drawn from the submitted sample, weighed and recorded in grams to three decimal places, and separated into three components: pure seed of the test species, seed of other species, and inert matter. Each component must then be weighed and recorded in grams to three decimal places. Calculation of the purity analysis must be done as follows:

The sum of the weights of the three component fractions of the submitted test sample must be compared to the original weight for any gain or loss. If a discrepancy of more than 5% of the original sample weight is found, the test must be discarded and a re-test is required.

The percentage by weight of each component fraction is calculated by the following formula:

$$\text{Component (\%)} = \frac{\text{weight of each component fraction}}{\text{Total test sample weight}} \times 100$$

$$\text{e.g. Pure seed (\%)} = \frac{\text{weight of pure seed fraction}}{\text{Total sample weight}} \times 100$$

When percentages of all three components are added together, they must equal 100%. The percentage by weight of pure seed must be expressed in one decimal place (e.g. 99.9%). In case the sum does not equal to 100% (either 99.9 or 100.1%), 0.1% must be added or subtracted from the largest value (usually the pure seed fraction). Fractions of the components less than 0.05% are recorded as 'trace'.

The pure seed fraction is used for seed weight determination and germination testing.

5.3 1000 Seed Weight Determination

This test determines the weight of 1000 seeds of a sample. Eight (8) replicates of 100 seeds must be drawn randomly from the pure seed fraction of the purity test. Each replicate weight is recorded in grams to three decimal places. The mean weight of 100 seeds must be used to calculate the weight of 1000 seeds. Variance, standard deviation and coefficient of variance must be calculated using the following formulas.

$$\text{Variance} = \frac{n(\Sigma x^2) - (\Sigma x)^2}{n(n-1)}$$

where :
x = weight of each replicate in grams
n = number of replicates, and Σ = sum of.

$$\text{Standard deviation, } s = \sqrt{Variance}$$

$$\text{Coefficient of variation, CV} = \frac{s}{\bar{x}} \times 100$$

where $\bar{x}$ = average (mean) weight of 100 seeds.

If the coefficient of variation exceeds 4.0, eight more replicates must be drawn and weighed. The standard deviation must be calculated for the 16 replicates and any replicate that diverges from the mean by more than twice the standard deviation must be discarded. The remaining replicate weights must be used to determine the weight of 1000 seeds.

5.4 Germination Testing

The objective of the germination test is to determine the germination potential of a seedlot. Percentage germination is defined as the percentage of seeds that develop into normal seedlings under specified conditions in a specified period of time.

Testing facilities must have and use the following materials and equipment:

- standard germination containers that allow for adequate and uniform spacing
- suitable paper substrate that has an open and porous nature and the capacity to hold sufficient water for the duration of the test period

- germination cabinets that can control temperature and light

Each test must consist of four hundred seeds which are drawn from the pure faction of seed and then randomly divided into four replicates of 100 seeds. Seeds for each replicate are placed on moist substrate in a germination container. Germination containers that hold multiple replicates cannot contain more than one replicate of the same seedlot. Refer to Table 2 for germination cabinet conditions, test periods for individual species, pretreatment requirements for stratified tests, and types of tests required. Germinants must be assessed according to vigour classes developed at Petawawa National Forestry Institute (see Figure 7 for seed vigour classes). For all species except *Populus* spp. and *Pinus albicaulis*, germinants are assessed at weekly intervals (days 7, 14, etc) during the specified germination test period. The number of germinants without abnormalities that reach vigour classes 1 and 2 are counted, recorded, and removed from the containers during the weekly assessments. On the final day of assessment all remaining germinants in vigour classes 1–7 are counted and recorded. The number of ungerminated seeds and the number of abnormal germinants, (i.e. albinos, multiple stems, stunted roots, dwarfed hypocotyls) must be counted and recorded on the last day of assessments. Aspen and balsam poplar germinants are assessed on days 3, 5, and 7, with the final count on day 10. Contact the Provincial Seed Officer for specific germination protocols for *Pinus albicaulis*.

Germination is expressed as the percentage of the normal seedlings reaching vigour classes 1 to 4 for each replicate within the germination test period. The overall germination percentage for a seedlot is calculated to the nearest whole number and is the mean of all replicates. The sum of the percentage of normal seedlings in all vigour classes, abnormal seedlings and ungerminated seeds must be 100. If the tolerance limits defined in Table 3 are exceeded, the germination test must be repeated.

Table 2. Germination testing prescriptions

Species	Temp (ºC)	Light (hrs)	First Count (days)	Final Count (days)	Pretreatment Requirements and Test Type
Abies balsamea	25	12	7	28	Pre-chill at 2-5°C for 21 days. Unstratified and stratified tests.
Abies lasiocarpa	25	12	7	28	Pre-chill at 2-5°C for 21 days. Unstratified and stratified tests.
Betula papyrifera	25	12	7	21	None. Unstratified test only.
Larix laricina	25	12	7	21	Pre-chill at 2-5°C for 21 days. Unstratified and stratified tests.
Larix sibirica	25	12	7	21	Pre-chill at 2-5°C for 21 days. Unstratified and stratified tests.
Picea engelmannii	25	12	7	21	Pre-chill at 2-5°C for 21 days. Unstratified and stratified tests.
Picea glauca	25	12	7	21	Pre-chill at 2-5°C for 21 days. Unstratified and stratified tests.
Picea mariana	25	12	7	21	Pre-chill at 2-5°C for 21 days. Unstratified and stratified tests.
Pinus albicaulis	Germination protocols under development				
Pinus banksiana	25	12	7	21	Pre-chill at 2-5°C for 21 days. Unstratified and stratified tests.
Pinus contorta	25	12	7	21	Pre-chill at 2-5°C for 21 days. Unstratified and stratified tests.
Pinus flexilis	25	12	7	21	Pre-chill at 2-5°C for 21 days. Unstratified and stratified tests.
Populus Spp.	25	12	3	10	None. Unstratified test only.
Pseudotsuga menziesii	25	12	7	21	Pre-chill at 2-5°C for 21 days. Unstratified and stratified tests.

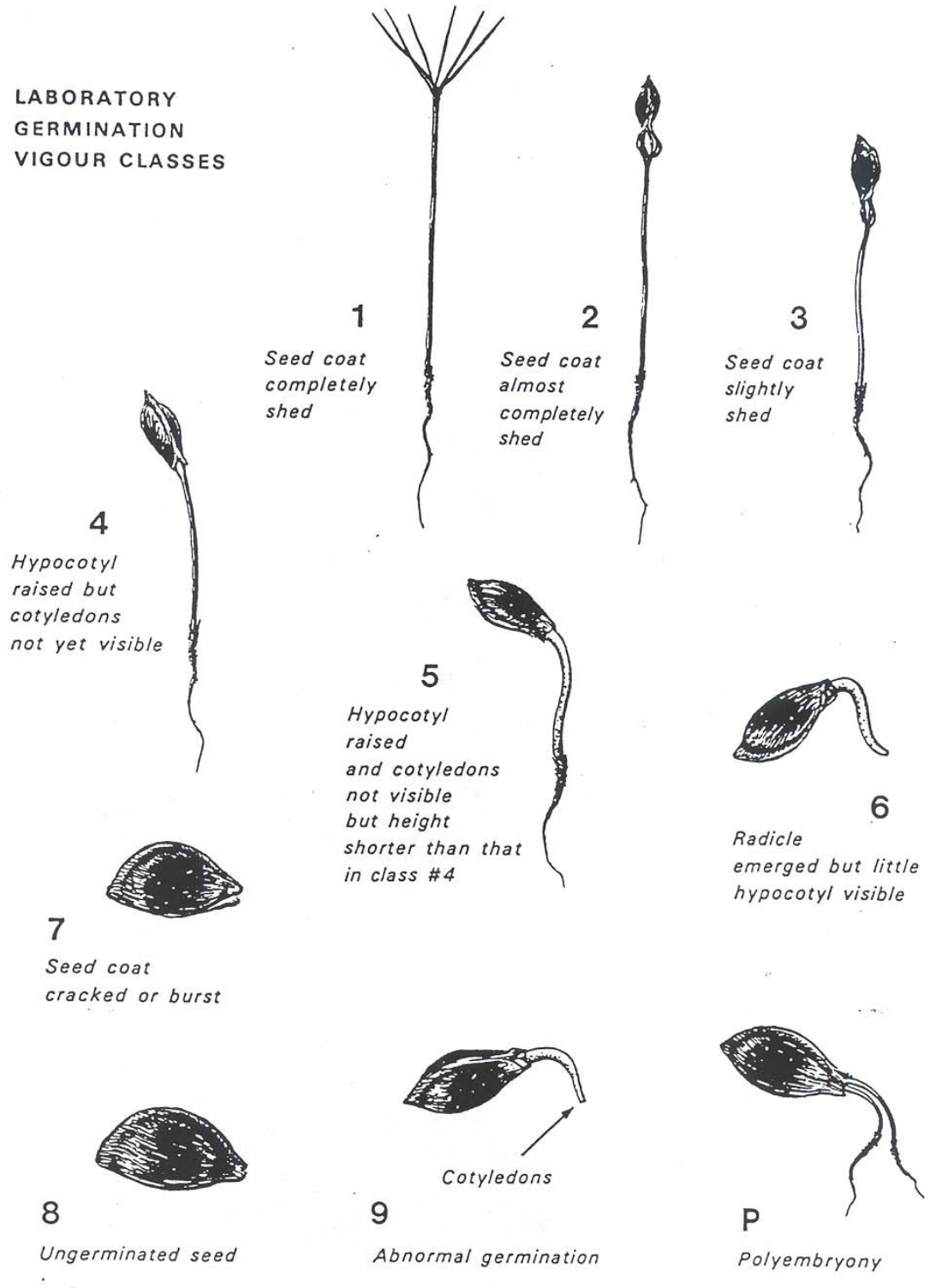
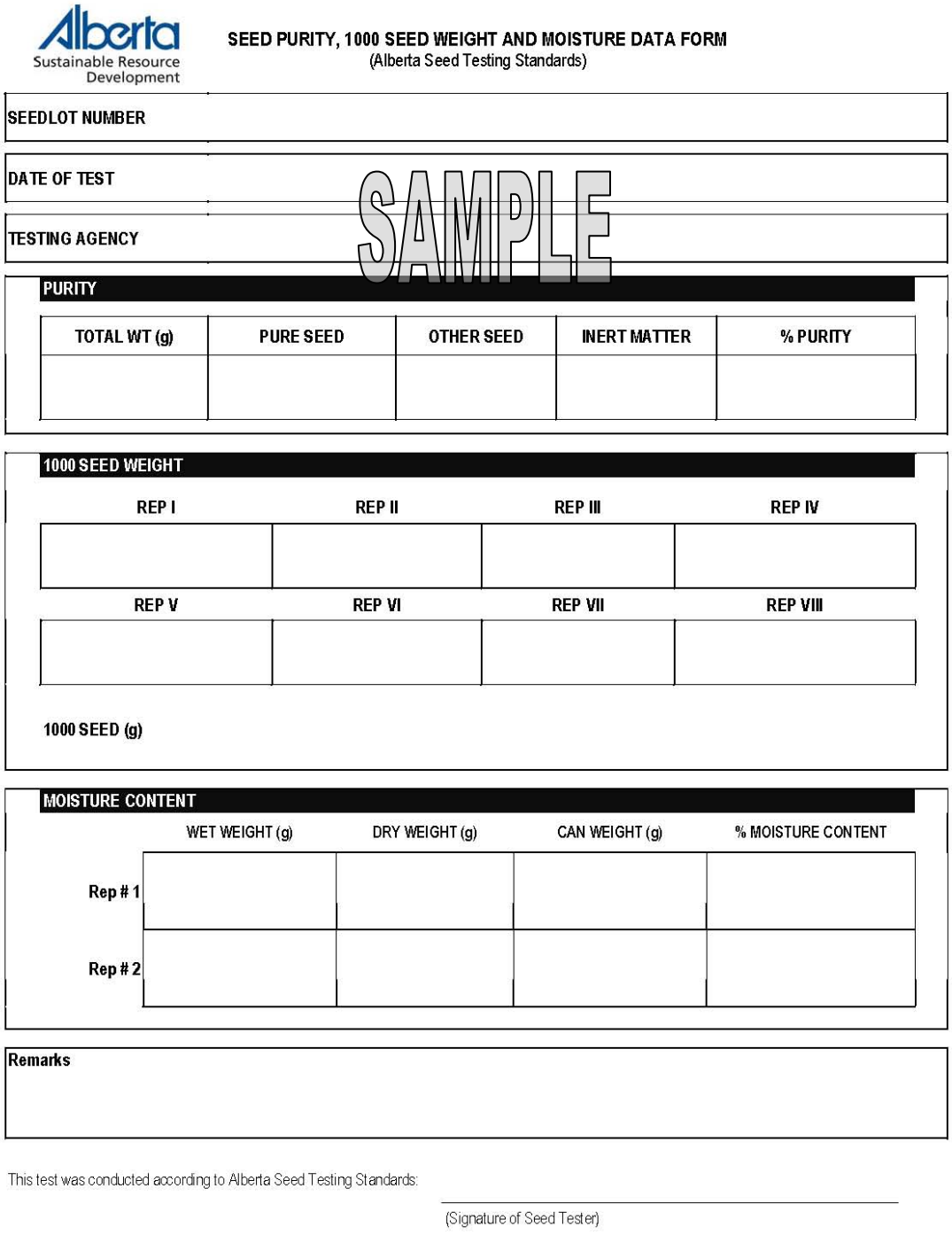


Fig. 1 Seedling vigour classes.

Table 3. Maximum range of tolerance between four replicates of 100 seeds in one germination test

If the average germination percentage		The maximum tolerated range
is:	or:	between replicates is:
99	2	5
98	3	6
97	4	7
96	5	8
95	6	9
93 to 94	7 to 8	10
91 to 92	9 to 10	11
89 to 90	11 to 12	12
87 to 88	13 to 14	13
84 to 86	15 to 17	14
81 to 83	18 to 20	15
78 to 80	21 to 23	16
73 to 77	24 to 28	17
67 to 72	29 to 34	18
56 to 66	35 to 45	19
51 to 55	46 to 50	20

To find the maximum tolerated range, calculate the average percentage germination of the four replicates to the nearest whole number. Locate the average in either of the first two columns and read the maximum tolerated range in the third column.



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SEED PURITY, 1000 SEED WEIGHT AND MOISTURE DATA FORM
(Alberta Seed Testing Standards)

SEEDLOT NUMBER

DATE OF TEST

TESTING AGENCY

SAMPLE

PURITY

TOTAL WT (g)	PURE SEED	OTHER SEED	INERT MATTER	% PURITY

1000 SEED WEIGHT

REP I	REP II	REP III	REP IV

REP V	REP VI	REP VII	REP VIII

1000 SEED (g)

MOISTURE CONTENT

	WET WEIGHT (g)	DRY WEIGHT (g)	CAN WEIGHT (g)	% MOISTURE CONTENT
Rep # 1				
Rep # 2				

Remarks

This test was conducted according to Alberta Seed Testing Standards:

(Signature of Seed Tester)

Fig. 2 Seed purity, 1000 seed weight and moisture content data form.

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GERMINATION TEST DATA FORM
(Alberta Seed Testing Standards)

SEEDLOT							Date						
Test Type (check one) ☐ Unstratified ☐ Stratified							# of seeds per rep						
Mean Germination %VC 1-7							Mean Germination %VC 1-4						
Range of germination percentage between replication (VC 1-4) at final count)													
Replication 1							**Replication 2**						
Vigor Class	Normal Removed - Day						Vigour Class	Normal Removed - Day					
	3	7	14	21	28	Total		3	7	14	21	28	Total
1							1						
2							2						
3							3						
4							4						
5							5						
6							6						
7							7						
Total							Total						
8							8						
Abnormal							Abnormal						
Remarks							Remarks						
Replication 3							**Replication 4**						
Vigour Class	Normal Removed – Day						Vigour Class	Normal Removed - Day					
	3	7	14	21	28	Total		3	7	14	21	28	Total
1							1						
2							2						
3							3						
4							4						
5							5						
6							6						
7							7						
Total							Total						
8							8						
Abnormal							Abnormal						
Remarks							Remarks						

This test was conducted according to Alberta SeedTesting Standards

Signature of Seed Tester

Fig. 3 Germination test data form.

6.0 References

International Seed Testing Association, 2005. International rules for seed testing edition 2005. International Seed Testing Association (ISTA), Bassersdorf CH-Switzerland

Edwards, D.G.W, 1987. Methods and procedures for testing tree seeds in Canada. Can. For. Serv., Victoria, BC. Forestry Tech. Rep. 36

Wang, B.S.P, 1976. Dormancy and laboratory germination criteria of white spruce seed. Proceedings, 2nd IUFRO International symposium on Physiology of Seed Germination, Fuji, Japan 1976, pp. 179-187