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Quality assessment methods for *Picea abies* seeds

Abstract: The quality assessment of spruce seeds consists mainly in determining their germination capacity, including germination rate (speed), but also purity and weight. Internationally recognised methods are listed in the ISTA Rules for Seed Testing which also specify test conditions. Some countries such as Poland use modified methods. Apart from providing information about the purity of a species, purity testing can also identify the proportion of winged seeds in each seed lot. Germination tests yield data on the maximum germination potential of a seed lot which are used to compare the quality of different lots and to establish the field sowing values. This paper presents the methods used for assessing the quality of Norway spruce seeds, and discusses their advantages and disadvantages and the problems that need further investigation.

Additional key words: Norway spruce, purity, germination, health testing

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Introduction

Seed quality assessment provides information about seed quality before and immediately after seed collection (e.g. degree of ripening and embryo development, proportion of empty and insect-damaged seeds), during and after handling procedures, throughout storage, and before sowing. The goal of seed testing in forestry is to provide reliable data on how many seedlings can be grown, and how many of them can thus be available for afforestation and reforestation.

Seed testing has been developed to minimise the shortage of adequate amounts of seeds and seedlings by assessing the quality of seed before sowing. Seed quality is a concept made up of different attributes that are of interest to different segments of the "seedling production industry": to the seed collector, the processor and warehouseman (in seed plants), the merchant, the nurseryman, and foresters at the end of the process. In all cases, the objective of doing a test is to determine the value of seed for planting. Since the behaviour of seed as a living biological product cannot be predicted with the certainty that characterises the test results of inert or non-biological materials, the testing methods must be based on the scientific knowledge of seed and on the accumulated experience of the seed analyst (ISTA Rules for Seed Testing, hereinafter referred to as the "ISTA Rules", 2003 ed.).

Internationally recognised methods are listed in the ISTA Rules. In some countries, however, modified methods are applied as a part of national rules (e.g. AOSA Rules in the U.S.A., or CSN 48 1211 = Czech Technical Rules in the Czech Republic). All these rules specify standardised methods which ensure the necessary accuracy and reproducibility. Using the ISTA Rules methods is of advantage to international trade as it enables laboratories in different countries to produce the same results within an acceptable range. Compared to agriculture, the volume of internationally traded seeds of forest trees and shrubs has been relatively small, and the standardised methods (either the ISTA Rules or others) have mostly been applied to evaluate the quality of seed used for domestic purposes (reforestation and afforestation).

This paper presents the methods used for assessing the seed quality of Norway spruce (*Picea abies* (L.) Karst.), and discusses their advantages and disadvantages and some problems that need further investigation.

Sampling

The objective of sampling is to obtain a sample of a size suitable for tests in which the probability of a constituent being present is determined only by its level of occurrence in the seed lot (ISTA Rules, 2006 ed.). In most cases, the seed owners are interested in the quality of the whole seed lot. Since only a working sample is tested, it must represent the entire seed lot. In agriculture, sampling has been commonly used in practice, and has been done in warehouses where seeds are handled and stored before trading. In forestry, representative sampling has been widely introduced with the OECD Scheme for forest reproductive material, and later within the EU countries via the Council Directive 1999/105/EC (2000). The Directive in Article 14, part 2 states:

"In the case of **seeds**, the supplier's label or document referred to in paragraph 1 shall also include the following additional information, assessed, as far as possible, by internationally accepted techniques:

a) purity: the percentage by weight of pure seed, other seed and inert matter of the product marketed as a seed lot;
b) the germination percentage of the pure seed, or, where germination percentage is impossible or impractical to assess, the viability percentage assessed by reference to a specified method;
c) the weight of 1000 pure seeds;
d) the number of germinable seeds per kilogram of product marketed as seed, or, where the number of germinable seeds is impossible or impractical to assess, the number of viable seeds per kilogram".

For Norway spruce this means that the quality of seeds should be evaluated after processing and before marketing. The supplier is responsible for providing the above information. However, besides the recommendation of using "internationally accepted techniques" there is no reference to whom should assess the seed quality: Should it be the seed owner (supplier) or any "independent" legal entity (e.g. laboratory)? In most countries there are "independent" laboratories testing seed quality, but seed sampling is done in different ways. According to the ISTA Rules, the validity of the results on the ISTA International Seed Lot Certificate is firmly linked to sampling and testing done by the accredited ISTA laboratories. In the Czech Republic, both the sampling and testing are provided by a laboratory accredited according the ISTA Laboratory Accreditation Standard (2007) and EN ISO/IEC 17 025:2005. Similarly, in Slovakia all marketed seeds are sampled and tested in an ISTA accredited laboratory. In Austria, testing is ensured by an accredited laboratory, but sampling is done by the seed owners.

Purity

The objectives of purity analysis are to determine (a) the percentage composition by weight of the sample being tested and by inference the composition of the seed lot, and (b) the identity of the various species of seeds and inert particles constituting the sample (ISTA Rules, 2007 ed.). There are three fractions to be determined: pure seeds, other seeds, and inert matter. The pure seeds shall refer to the species stated by the applicant, or found to predominate in the sample.

The first step in purity analysis is to identify the tested species. In Norway spruce, as in most other conifer seeds, after processing, cleaning and dewinging it is difficult or impossible for the analyst (using lens or a stereomicroscope) to identify with certainty the seed species even if the seed sample contains winged seeds. Then, only the genus name of the tested seeds is reported on the certificate according to the ISTA Rules. Recently, the same approach has been used in the Czech Republic and has been incorporated into ČSN 48 1211 (2006) for testing the quality of forest tree seeds. And it does not matter that the master certificate states the species because the result of purity analysis has been based only on laboratory evidence. Some software programs have been developed with a suitable database enabling determination of agricultural species. In the future, such programs used in conjunction with microscopic examination could also help to identify tree species.

For determination of the portion of pure spruce seeds, the ISTA Rules provide so-called "pure seed definition" (PSD) No. 47 which reads as follows:

"Seed, without wing or integument, provided a portion of the testa is attached. Piece of seed larger than one-half the original size, without wing or integument, provided a portion of the testa is attached.

N.B. 'Integument' refers to the tissue attaching the wing to the seed. In *Pinaceae* with this definition, the integument is not intimately associated with the seed and is usually removed in processing thus removing the wing. However, if an integument (with or without wing) is still attached to any seed during the purity analysis such seed will be regarded as 'winged seed' and must be left intact – neither the integument nor wing should be deliberately removed. Winged seed (i.e. seed with an attached integument with or without a wing of any size) must be weighed and reported as a separate percentage from 'pure seed' according to paragraphs 3.5.2.A.9 and 3.7. After weighing, the winged seed and pure seed fractions should be recom-

bined and used in representative proportions for counting out the germination replicates".

So, in fact, spruce seeds with and without wings are considered as pure seeds. The determination of the portion of winged seeds provides suitable and essential information for forest nurseries using precise sowing machines. The Czech PSD, used for many years, defines as 'pure spruce seeds' only those without wings. In purity analysis, the wings are manually removed and added to the inert matter. This procedure can be time-consuming (even wasting) in the case of poor-purity seed lots, and provides limited information about seed lot quality. Fig. 1 shows the results of purity tests according to the ISTA Rules and the Czech Technical Rules on such poor-quality seed lots.

However, pure seed also includes a "piece of seed larger than one-half the original size" even if such damaged spruce seed cannot germinate (some important parts of embryo are missing). So, recently, a debate about possible revision of this statement has been started by Dr. G. Edwards within the ISTA Forest Tree and Shrub Seed Committee.

Weight determination

The purpose of this simple test is to enable calculation of the number of seeds in a certain weight (e.g. 1 kg) of the material. For Norway spruce, this has mostly been done by manual counting of eight replications (8 × 100 seeds) to determine their mean weight. The use of counting machines is also possible but, in contrast to e.g. corn seeds, Norway spruce seeds in a sample can significantly differ in size, which can result in incorrect numbers.

Germination

The germination test aims at determining the maximum germination potential of a seed lot, which can then be used to compare the quality of different lots and to estimate the field sowing value. The test conditions are controlled to ensure the most regular, rapid and complete germination for most samples of a particular species (ISTA Rules, 2003 ed.). As the test is focused on determining the maximum germination, the results of seed emergence under the field conditions (mainly in bare-root nurseries) can widely differ from the laboratory results. However, in container nurseries (in greenhouses) with controlled temperature, sufficient irrigation, and suitable substrate, the laboratory results predict the emergence with a high probability.

The germination test is the most important assessment of seed quality. Besides providing data about the maximum germination capacity, it also gives information about seed vigour. Norway spruce seeds

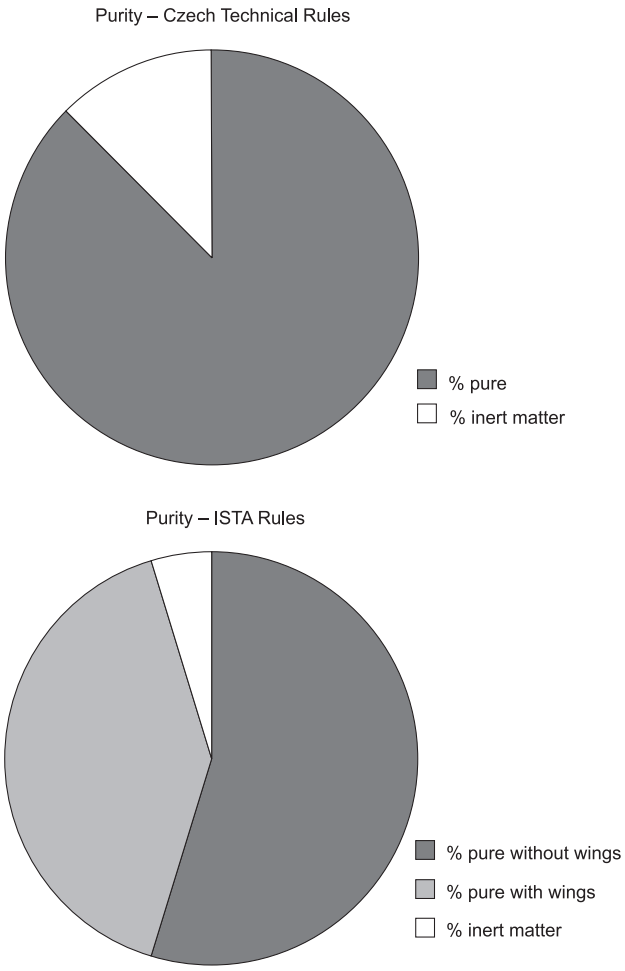

Fig. 1. Results of Norway spruce seed purity tests according to Czech Technical Rules and ISTA Rules

are planted on top of paper substrate (filter, blotter, creped cellulose paper without a blotter or towel paper) which should ensure sufficient water supply and prevent the roots from growing into the paper. The general quality of the paper substrate and water has been defined for quite a long time, but recently there are more precise requirements specified in the ISTA Rules (2006 ed.): pH within the range 6.0–7.5 when checked in the substrate; conductivity – the salinity must be as low as possible and no more that 40 milli-Siemens per metre; cleanliness and innocuousness – the substrate must be free of harmful fungi, bacteria, seeds or toxic substances. The alternative allows either purchasing the growing medium from commercial suppliers with the requested specifications or replacing the measure of conductivity with a biological test for phytotoxicity.

The water should have the same pH value as the substrate (6.0–7.5). So, in some laboratories distilled, demineralised, de-ionised water is used when tap water does not meet the requirement. However, if evidence based on statistical data proves no influence on the germination test results, water with pH outside the range can be used.

The prescribed temperature for germinating Norway spruce seeds is either alternate or constant. The ISTA Rules (2006 ed.) specify a temperature of 30°C for 8 hours in the light alternating with 20°C for 16 hours in the dark. Another possibility is seed incubation at constant 20 or 25°C (AOSA 2006). In Poland, for a shortened informative test, Norway spruce seeds can germinate at constant 24°C (Załęski et al. 2006). Some seed lots react better to alternating temperature while others get better results if a constant temperature is used. Some laboratories use thermogradient tables to determine both germination and vigour based on the results of comparative germination tests at several temperature regimes. The test results predict better the behaviour of a seed lot in non-optimum field conditions. In Sweden (Prescher and Prescher 2003), seeds are tested for sensitivity to different temperatures during the germination phase. This information is very important for the nursery. Sowing in Swedish container nurseries is done three times during the year, in mid-March, at the end of May, and at the beginning of July. The same seed lot can be sown at all three times, so that the seed should be as adaptable to different germination temperatures as possible. In March, there can be a problem with maintaining warmer temperatures during cold nights, while in May it is not unusual to have summer conditions in southern Sweden. Adaptability is especially important during the last sowing in July, when the temperature in the greenhouse can be more or less out of control during hot summer days. Consequently, the Swedish started using thermogradient tests some 20 years ago, and today such a test is standard for all seed lots used in container nurseries. The test is performed by studying the germination rate at temperatures ranging from 15 to 30°C. However, this method can be used only when a limited number of seed lots is tested because of some space and equipment requirements.

The duration of the germination test for Norway spruce seeds varies between 21 days (e.g. ISTA Rules, 2005 ed.; CSN 48 1211 2006) and 16 days (AOSA 2006). In Poland, in the shortened (informative, non-official) test, the germination of Norway spruce seeds takes only 14 days (Załęski et al. 2006). The rules usually prescribe that the first counting of germinants (both for spruce and most other woody species) should be done after 7 days. This first count gives information about the speed of germination that can express the seed lot vigour. When we compare germination speed in Norway spruce and Scots pine (*Pinus sylvestris* L.), the majority of pine seed lots germinate faster than the spruce seeds. While in Scots pine the germination rate (speed) is usually highest after 7 days, seeds of Norway spruce reach a germination peak mostly after 10 days (Prescher and Prescher 2003; Procházková 2007). According to the ČSN 48 1211 (2006) Rules, the germination of Norway spruce seeds is recorded after 7, 10, 15, and 21 days, while in Sweden it is done after 7, 10, and 21 days (Prescher and Prescher 2003). Two Swedish laboratories, testing forest tree seeds according to the ISTA Rules, calculate the ratio "10 days/21 days" for Norway spruce seeds that is far more relevant than the "germination rate at 7 days/21 days" (Prescher and Prescher 2003). The Preschers state: "One has to remember that the measure of the germination at day 7 and 10 respectively could be difficult, since the slope of the accumulated curve is very steep. Even small changes in the germination environment can result in displacement of the germination curve, which affects the ratio "7d/21d" or "10d/21d" to a great extent. It could be so sensitive that the counting of germinants should be done at the same hour of the day as the germination test started. For these reasons it is difficult to compare results of the ratio, and tests should, if comparison is necessary, therefore be made at the same laboratory under the same germination conditions (Sahlén and Henriksson 1985 ex Prescher and Prescher 2003)".

Generally, the percentage germination reported on test certificates indicates the proportion by number of seeds producing normal seedlings under prescribed conditions and within a specified period. Usually, normal seedlings should have developed all their essential structures as described in the various rules. In tree seeds with epigeal germination, such as Norway spruce, a seedling is considered normal when the primary root and hypocotyl together are four times the length of the seed, provided that all structures which have developed are intact (ISTA Rules, 2003 ed.). At this stage the analyst should be able to recognise abnormalities in seedling development. In fact, distinguishing normal from abnormal seedlings can be done at an earlier developmental stage when the radicle reaches just the length of the seed. However, "the four times" rule results in better information about seed vigour.

Besides assessing normal and abnormal spruce seedlings, germination tests identify other seed categories: hard, fresh, and dead seeds, and by client's request also empty and insect-damaged seeds. The occurrence of fresh seeds provides information about dormancy level. Even if prechilling (at about 0–5°C) is not demanded for Norway spruce, the majority of Norway spruce seed lots react to chilling by higher uniformity and faster germination and emergence. The latter information could be useful for container nurseries. In some laboratories, such as the Forest Tree Seed Centre Laboratory (BC, Canada), the procedure for spruce germination includes 24-hour soak before the germination test itself (Kolotelo et al. 2001).

Health testing

Common saprophytic or weakly pathogenic fungi (*Aspergillus*, *Penicillium*, *Trichothecium*, *Chaetomium*, and many others) and pathogenic fungi (e.g. *Fusarium*, *Cylindrocarpon*) can colonise and infect Norway spruce seeds. The fungi which can occur on spruce seeds during germination tests can be detected after the incubation of the seeds on common agar substrate. There is only one standardised and validated method for detecting a pathogen (ISTA Rules, ed. 2005, Annexe to Chapter 7: Seed Health Testing Methods). The pathogen is *Caloscypha fulgens* (Pers.) Boud (anamorph *Geniculodendron pyriforme* Salt) which infects seeds of different spruce species, mainly *Picea glauca* and *P. engelmannii*, but also Norway spruce. *Caloscypha fulgens* is a seed-borne pathogen which attacks seeds before they germinate. Once the germination begins, the seeds are not susceptible to infection. The affected seeds get mummified rather than rotted, thus the seed lots suspected of being infested should be assayed. Seed lots that germinate poorly following stratification (when fungus spreads) are prime candidates for assay (Sutherland et al. 2002). Another seed-borne pathogen also affecting *Picea* spp. seeds is *Sirococcus conigenus*. Sutherland et al. (2002) describe methods for isolating several seed-borne pathogens including *Caloscypha fulgens*, *Sirococcus conigenus*, *Fusarium*, *Cylindrocladium*, and other fungi.

Validity of results

The ISTA Rules state generally that tests results are valid for one month. It is not permissible for the same seed-testing laboratory to issue another certificate (of the same colour) as a result of a further sampling from the same seed lot within one month of the previous sampling or to approve such sampling (ISTA Rules 17.6). While the purity and weight determination results of both fresh and stored seeds are the same (when the sampling is proper), the germination capacity and especially germination rate change during storage. Some national rules restrict the validity of germination test results: according to CSN 48 1211 (2006), the validity for Norway spruce is 12 months; in Canada the retesting of spruce seeds must be done after 24 months. The validity of germination results provides therefore important information that should be indicated on the seed lot label.

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