Successful Trial With Innovative Cold NSure Test on Douglas-fir Seedlings

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Forest tree nurseries rely on a tight scheduling of operations to be able to deliver vigorous seedlings to the planting site. Refrigerated storage is used to maintain planting stock in an inactive condition and to ensure adequate stock availability for geographically distinct planting sites. Cooler and freezer storage has therefore become common practice, but can present challenges. Lifting and storage of insufficiently hardened plants reduces vitality and may lead to cold damage, dehydration, and fungal infection. To prevent this kind of damage, and its adverse economic effects on nurseries and their customers, it is of vital importance to be able to accurately determine seedlings' peak physiological condition for harvesting, storage, or shipping to the field.

The NSure Cold Hardiness Assay

Last year, a new method to measure cold tolerance was introduced by NSure, a spin-off company from Wageningen University in the Netherlands. The test is based on measuring the level of activity of a carefully selected set of genes. Because all physiological responses are started and orchestrated by genes switching on or off, this method can be highly accurate and reliable. In fact, a comparable technology has been used in medical diagnostics for some time now, predominantly for making complex diagnoses such as tumour typing. NSure developed a sampling procedure that enabled the application of this technique in agro-production, because no lab is required.

In the Summer 2006 issue of FNN, an article described this new technology. At that time, NSure had tests available for Scots pine *(Pinus sylvestris),* Norway spruce *(Picea abies),* and European beech *(Fagus sylvatica).* The assay is based on the relative activity of 3 indicator genes that together provide enough information to give an estimate of the cold hardiness stage of the seedling. The corresponding genes dominate

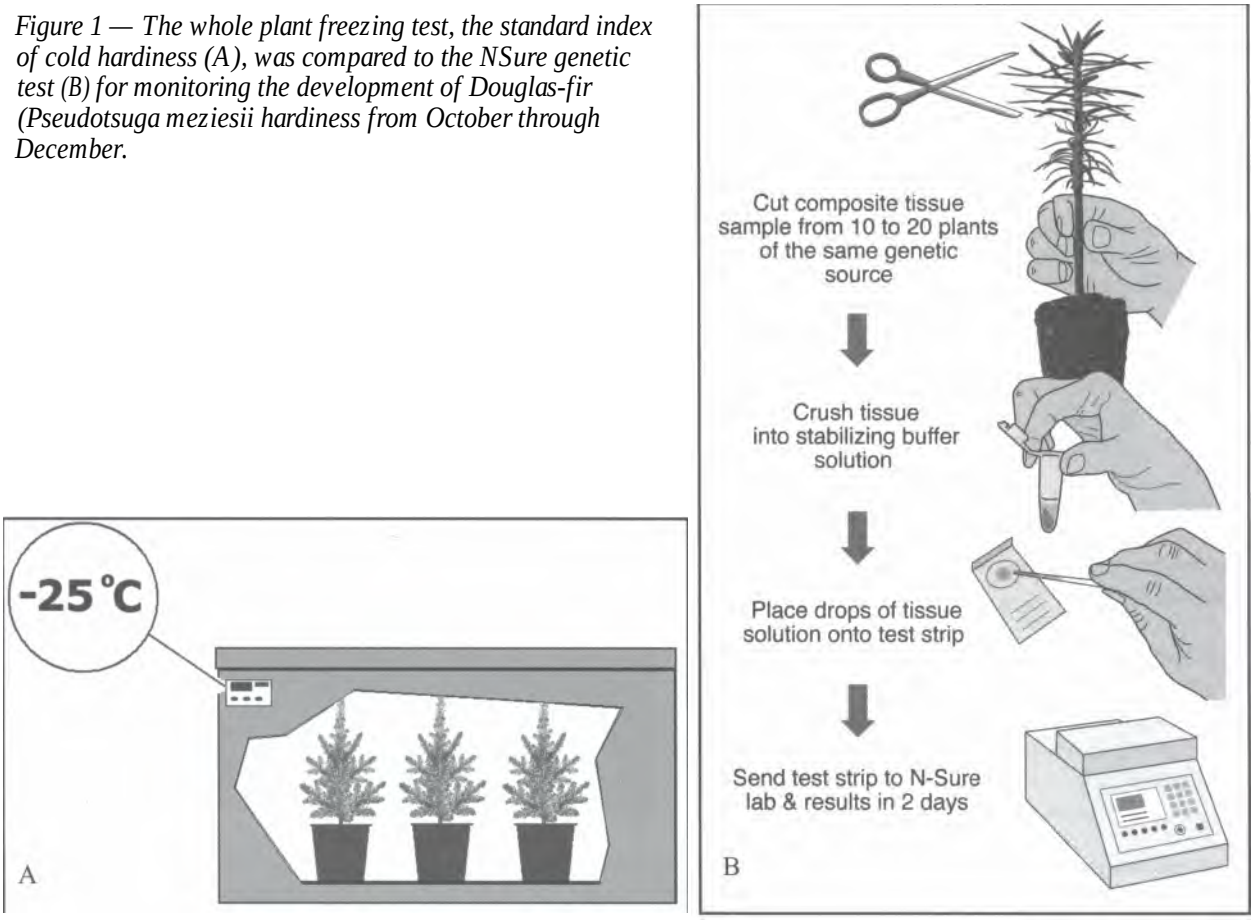

Figure 1 — The whole plant freezing test, the standard index of cold hardiness (A), was compared to the NSure genetic test (B) for monitoring the development of Douglas-fir (Pseudotsuga meziesii hardiness from October through December.

the process of hardiness development in all provenances studied and have a strong positive correlation with shoot electrolyte leakage (SEL) cold hardiness tests. The activity of three of these indicators (2 differentially regulated dehydrin gene family members and one control gene) is measured in the cold hardiness test implemented by the company NSure.

Testing Douglas-fir

Because nurseries from the Pacific Northwest region were interested in this technology, NSure developed a new assay aimed at one of the economically most important species in this region: Douglas-fir *(Pseudotsuga menziesii).* The NSure test was been examined during the 2006-07 season as part of a larger cold hardiness project with the Nursery Technology Cooperative (Dept. of Forest Science, Oregon State University).

Cold hardiness at time of lifting was compared between the Whole Plant Freezer Test (WPFT) and the NSure test across 5 dates (October through December, 2006) for 6 different Douglas-fir seed lots. For the WPFT, seedlings were placed into a programmable chest freezer at 4 target temperatures (Figure **1A).** After freezing, seedlings were placed into a greenhouse with optimal growth conditions for 6 days and then assessed for foliar, bud, and cambial damage to estimate the LT_{10} or LT_{50} (lethal temperature to 10% or 50 % of the seedlings, respectively).

The NSure test was conducted on needles and buds collected from the same seedlings used for the WPFT test. The tissue was processed according to the sampling protocol provided with the Nsure sampling kit. Samples were then sent to The Netherlands for analyses. NSure measured the level of expression of 6 indicator genes and calculated the corresponding hardiness status using models derived from *P. sylvestris and P. abies.*

Results

The results indicate a typical development of cold tolerance as the autumn proceeded as well as differences between seed lots derived from different elevations. The stages of frost tolerance that are distinguished by the NSure method corresponded to different levels of frost tolerance as measured using the WPFT technology.

The NSure assay distinguished 3 stages of frost tolerance. These phases were shown to correlate very well with the **LT** values from the whole plant freezing test (Table 1).

When needles were used, however, the correlation was poor. In contrast to previous findings with Norway spruce and Scots pine, this study indicates that *Pseudotsuga* needles may not be as reliable as test material.

Based on these results the NSure method seems to be a good alternative for cold hardiness assay. The big advantage of the NSure test is that seedlings do not have to be transported to a test laboratory. The samples can be taken and stabilized on site. Any physiological changes occurring in response to transport conditions will therefore not hamper the outcome of the test.

Future Testing

NSure would like to extend the present dataset with samples from the 2007-08 season. Nurseries growing Douglas-fir are therefore encouraged to try the ColdNSure assay this year at a considerably reduced price. A 50% reduction is offered to nurseries that are willing to share information on batch quality with NSure. Especially results of alternative cold hardiness measurements performed on the same batch would be very valuable to NSure.

Table I—Comparison of the NSure assay of Douglas-fir buds and the whole plant freezing test for cold hardiness

NSure Phase	Cold Hardiness from WPFT Measured by LT50	Nursery Application
0	No frost tolerance observed	Seedlings are not hardy
1	23 and 14 °F (-5 and **-10** °C)	Developing hardiness—adequate for short-term cold storage: 33° to 36°F (**1**° to 2°C)
2	Below 14 °F (-10 °C)	Developing hardiness—adequate for long-term freezer storage: 30° to 25 °F (-2° to -4°C)

At present, NSure test facilities are located in Sweden and The Netherlands. Samples must be sent to one of these locations for analyses which will lengthen the response time. NSure is looking for partners, however, to set up a local test lab that will be able to generate a result report within 1 or 2 days after receipt of the sample.

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