

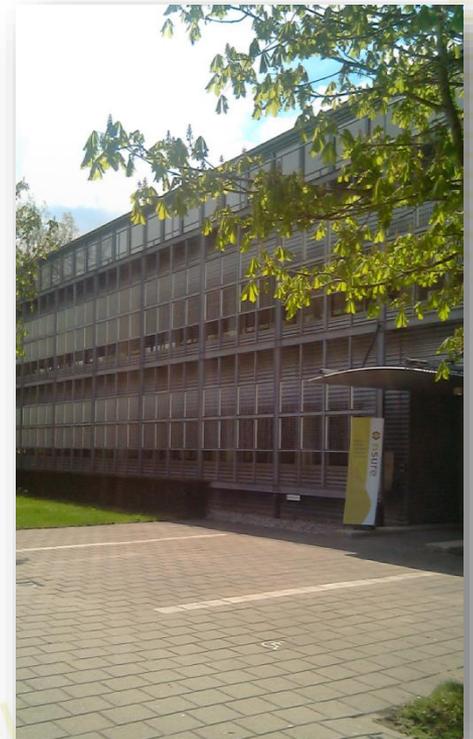


Listen to the genes

The use of molecular diagnostics to improve the quality of forestry seedlings

About NSure

- Private company since 2006.
- Spin-off from Wageningen University.
- Dedicated to optimize production and reduce waste for arable crops, vegetables, fruits, ornamentals, and trees.
- Specialized in converting gene activity data into practical tools.



NSure's technology

Focus on gene activity analysis



- *Plant senses change in conditions*



- *Related genes switched on / off*



- *Changing RNA content*



- *Changing protein content*



- *Changes in chemical composition*



- *Effect on physiology / quality*



**NSure's
measuring
point**

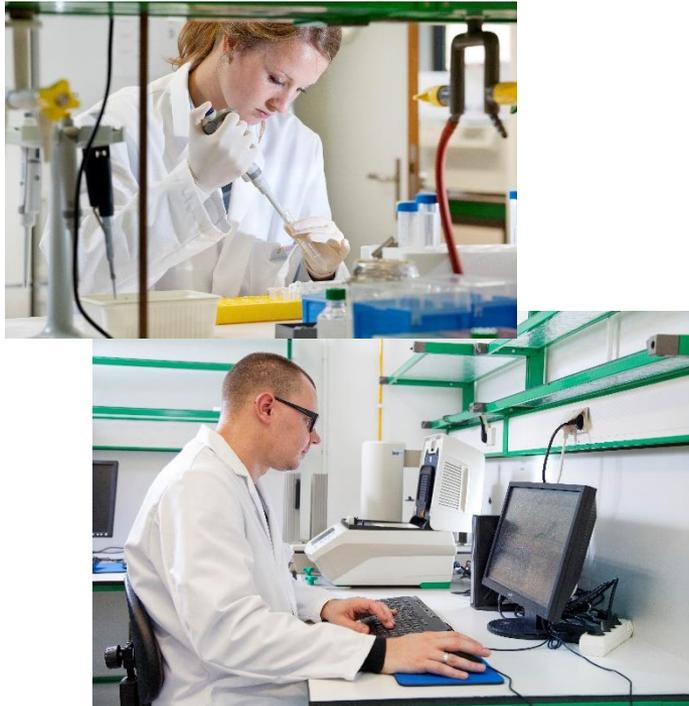


**Traditional
measuring
point**

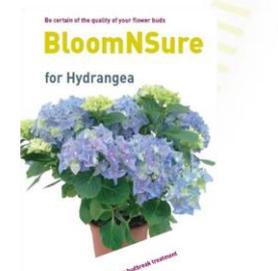
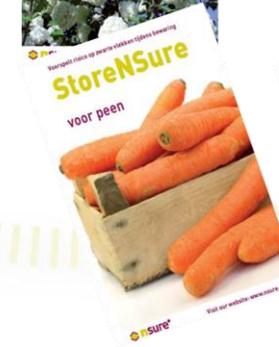


Applications of NSure technology

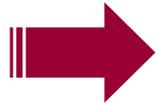
Contract research



Tailormade tests
(development and analysis)



NSure's tests



- **ColdNSure** - determines if forest tree seedlings are ready for frozen storage
- **BreakNSure kiwi** – determines the optimal moment to apply a bud breaking agent
- **FreshNSure** – determines whether a batch of apples/pears has been treated with 1-MCP (slows down ripening)
- **StoreNSure Strawberry** – determines when tray plants are ready for lifting
- **BloomNSure Hydrangea** – determines the earliest moment of cold storage
- **BloomNSure Viburnum** – determines if bud dormancy has been released

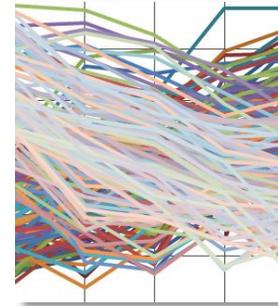
How do we develop a test?



Take samples



RNA sequencing

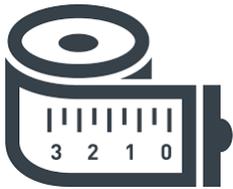


Compare the activity of all genes between the samples

1 2 3 4 5



Collect physiological data

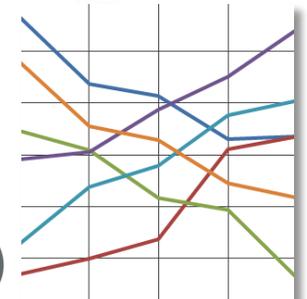


Reveal correlations, identify relevant genes



The Test

Analyse the activity of individual genes (qPCR)

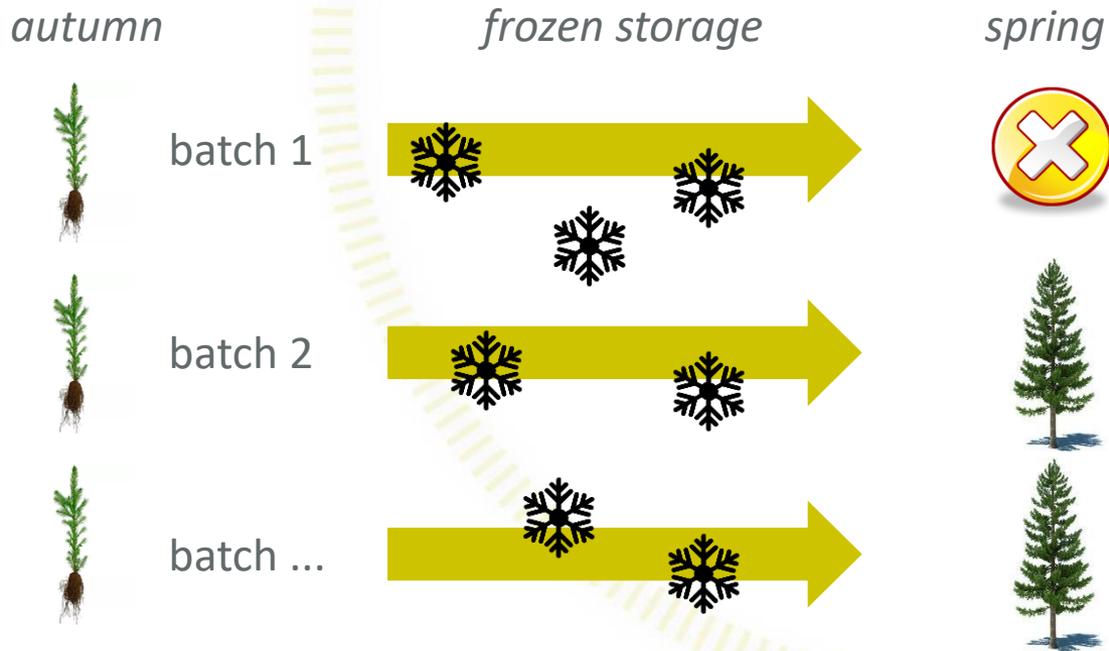


1 2 3 4 5

Molecular diagnostics to improve forestry seedling quality

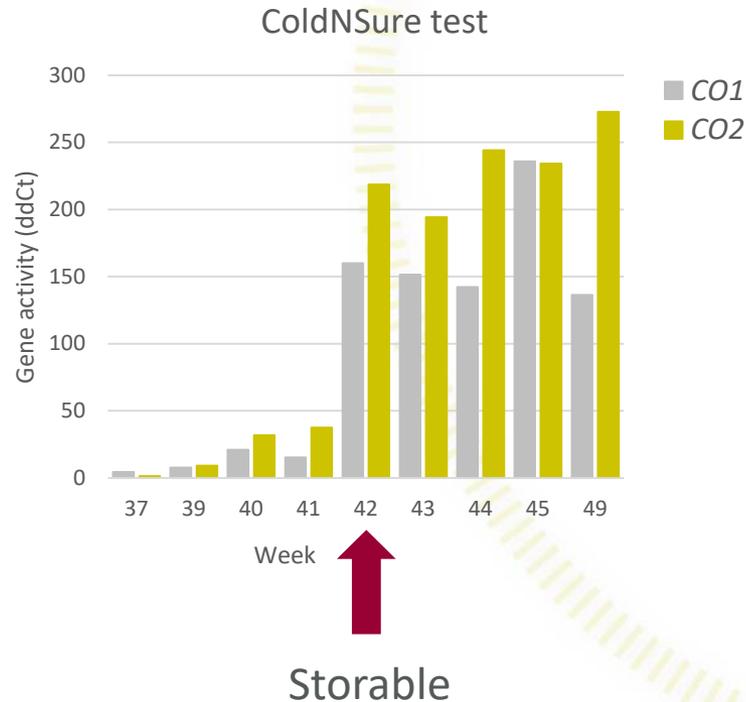
- Seedling quality plays an important role in the success of reforestation.
- Nurseries have to make irreversible decisions during the cultivation of seedlings which can have a major impact on seedling vitality.
- It is essential that nurseries have access to reliable methods that can help them.
- The ColdNSure test was initially developed for Swedish nurseries, but now it is also marketed in other European countries.

ColdNSure: determination of freezing tolerance in tree seedlings

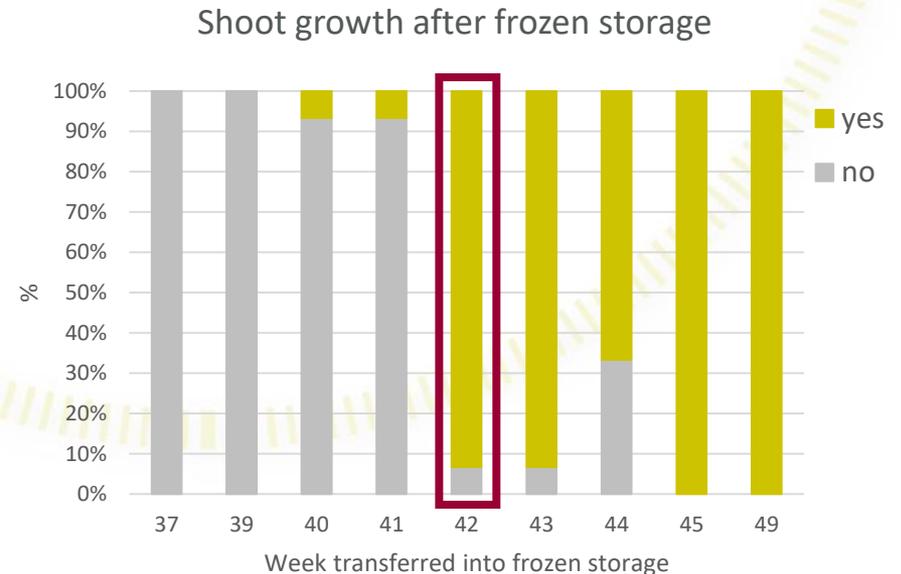


Available for 1-2 year-old seedlings of *Picea abies* (Norway spruce), *Pinus sylvestris* (Scots pine), *Pseudotsuga menziesii* (Douglas fir).

ColdNSure: determination of freezing tolerance in tree seedlings



Picea abies,
1.5 year old seedlings



Measurement is based on the activity of a set of carefully selected genes, mainly involved in low temperature protection.

From sample to result



Sampling kit

1 Select seedlings

- a** Pick 15 seedlings from a batch. A batch is defined as seedlings from a single sowing date and seed batch growing in the same plot or greenhouse.
- b** Important: don't let the seedlings dry or in the dark before sampling. This will influence the result.
- c** Take seedlings randomly from the growth area, both from the inside and the borders of the plot.
- d** Select seedlings without disorders or deficiencies. The seedlings should represent the average of the batch. Do not select seedlings that show deviant morphology or that differ in length from the average of the batch.
- !** Continue to step 2 within 1 hour, using following protocol:

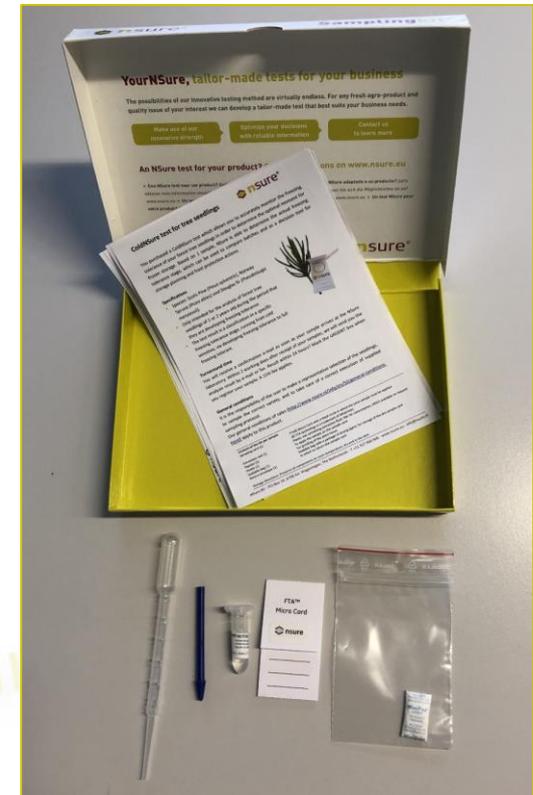
2 Make juice sample

-  Take the top part of the 15 seedlings.
-  Dissect the apical bud from the seedlings. Scales can be included. For large buds only use the top part.
-  Combine all buds or parts of buds in the tube.
-  Grind the buds using the micro-pestle. Make sure all buds are crushed. Mash max. 1 min!
-  Collect some juice from the tube using the pipette.
-  Apply 2 drops of juice inside the circle on the sampling card.
-  Air-dry the card for at least 1 hour. The card must be completely dry.
-  Insert dried card into the grip seal bag with drying agent.

3 Register & Send

- a** Log into your MyNSure account www.nsure.eu.
 - b** Click on 'Sample Registration' and complete your sample details.
 - c** No internet? Please contact us by phone +31 317 466 666 or fax +31 317 413 322 before sending the sampling card to the lab.
 - d** Send dried sampling card to NSure in provided envelope.
 - e** Results will be reported within 2 working days after receipt of your sample card. This return time is not guaranteed for not registered samples.
- Results within 24 hours? Mark the URGENT box while registering your sample (15% extra charged).
- More information? Go to www.nsure.eu or call +31 317 466 666. We will be glad to help you!

Procedure

Easy sampling method



Randomly collect 15 seedlings from the field and collect the top bud.



Transfer the buds in the tube.



Crush the buds with a pestle.



Suck up some juice with a pipet.



Apply 2 drops of juice on the sampling card.

Let the card dry.



Analysis and reporting procedure



Dried sampling card arrives at NSure



qPCR analysis

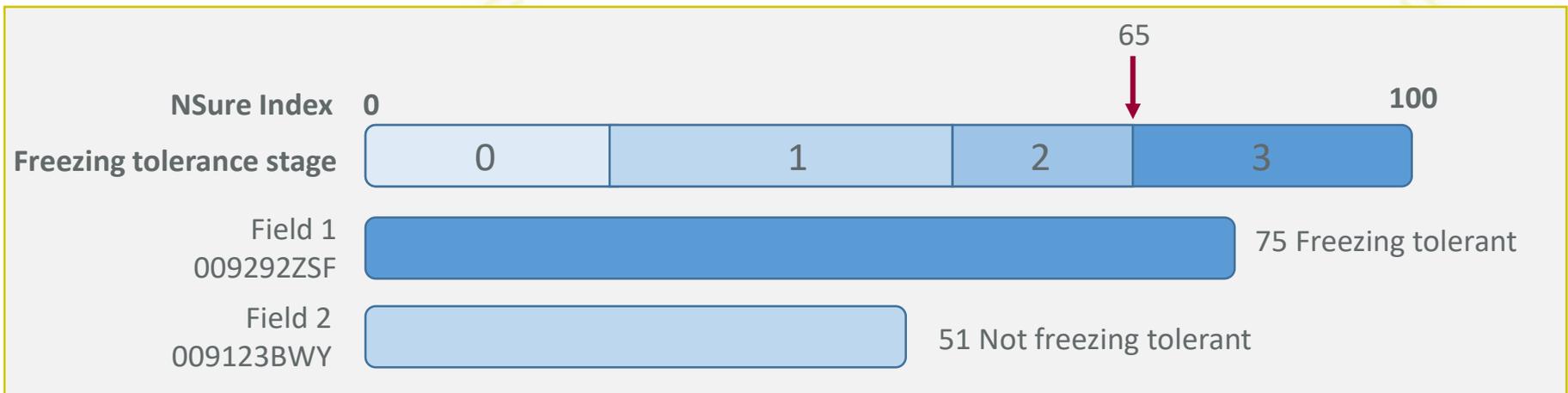


Results interpreted by NSure and reported within 48 hrs (urgency 24 hrs)

Report

- NSure defines four stages of freezing tolerance:
 - Cold sensitive (0)
 - Developing freezing tolerance (1) – early signs of freezing tolerance can be recognized
 - Developing freezing tolerance (2) – Approaching full freezing tolerance
 - Freezing tolerant (3)

- For *Picea Abies* (Norway spruce), you can even monitor the level of freezing tolerance more precisely, using the NSure Index (freezing tolerant ≥ 65).



The use of the ColdNSure for research purposes



A short day treatment leads to earlier frozen storage

- An important key attribute in the cultivation of Norway spruce is the dormancy status.
- The dormancy cycle is affected by seasonal changes in day length and temperature.
- Nurseries in Sweden perform a short day (SD) treatment in summer to:
 - inhibit plant growth
 - promote bud set
 - induce dormancy

A short day treatment leads to earlier frozen storage

- SD treatments are conducted in the greenhouse or in specially equipped areas outdoor.
- The intensity and durations of SD treatments vary widely across regions and nurseries (8-12 hrs in 4-35 days).
- What is the impact of a SD treatment in summer on the development of freezing tolerance?

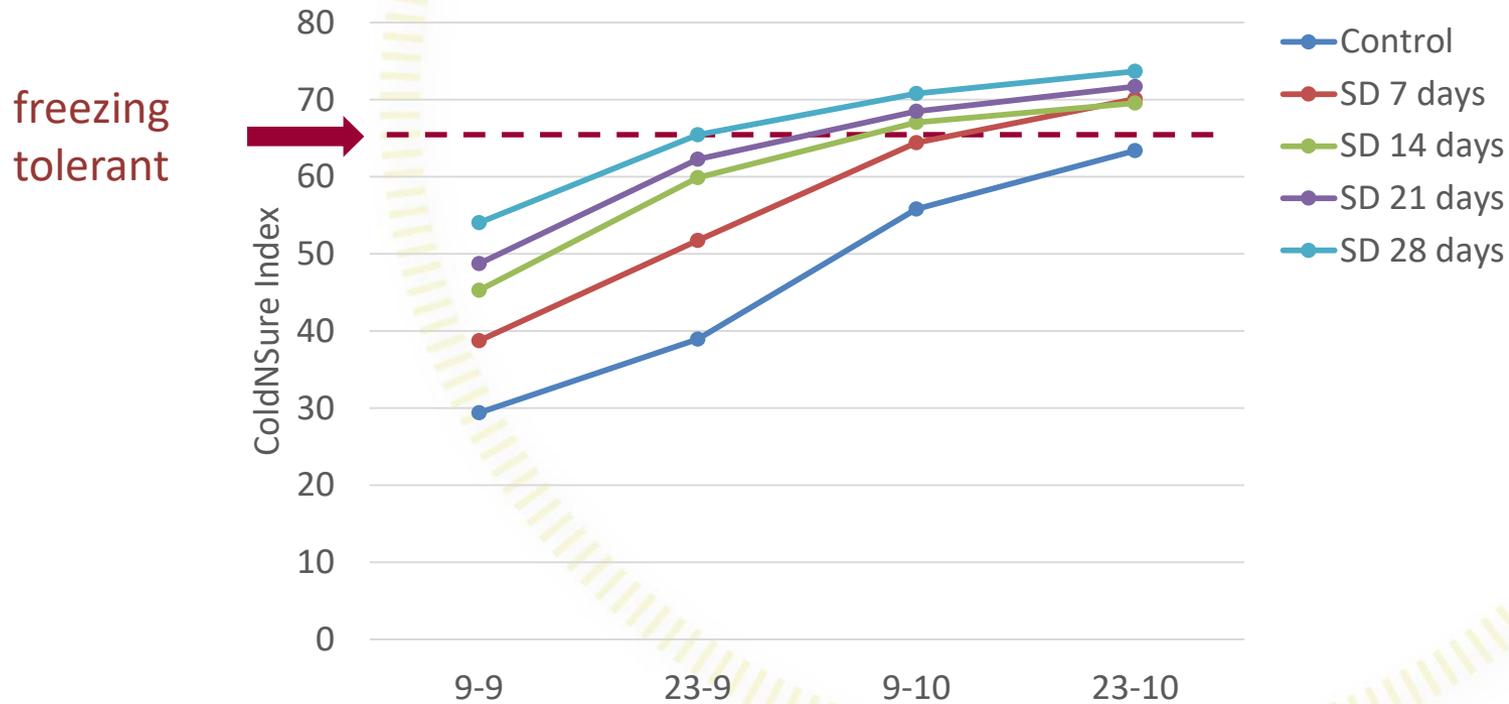


Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Forest Sciences



A short day treatment leads to earlier frozen storage



- SD treated seedlings are earlier storable!



Summary

- Molecular tests open up possibilities to forecast plant reactions to environmental changes.
- With help of the ColdNSure you can determine when your seedlings are ready for frozen storage.
- The test is based on measuring the activity of carefully selected set of genes which are involved in the development of freezing tolerance.
- A grower can perform the sampling directly in the field using the easy to use sampling kit.
- Upon arrival of the sample, results are reported within 48 hrs (urgency 24 hrs).
- A SD treatment in summer leads to earlier frozen storage.



Thank you for your attention

- Give us a call **+31.317.466666**
- Send us an email **info@nsure.nl**
- Visit us in Wageningen or at **www.nsure.eu**