Freeze acclimation and deacclimation

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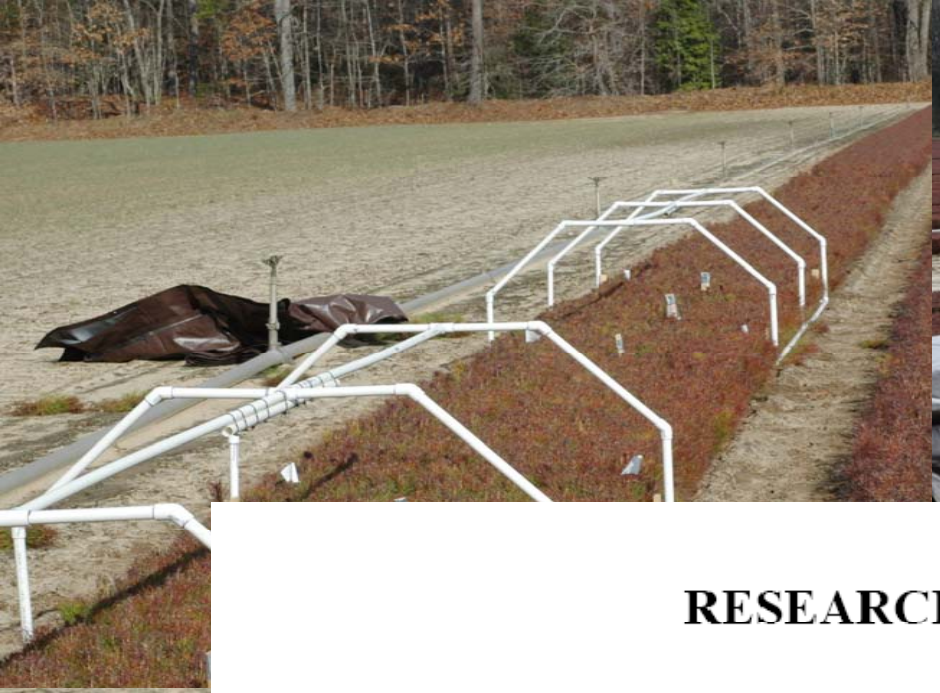
Three studies

- Deacclimation study 2006-7 VA – TN study
- Deacclimation study 2007-8 NC – VA study
- Cold Nsure study 2008 VA – AL study

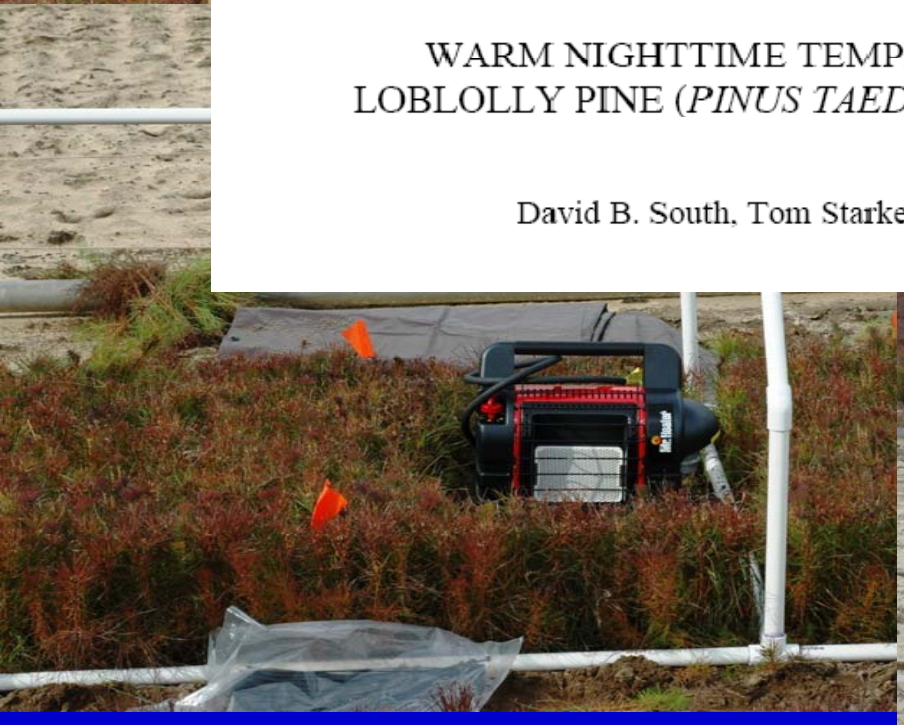
Deacclimation study 2007
VA and TN

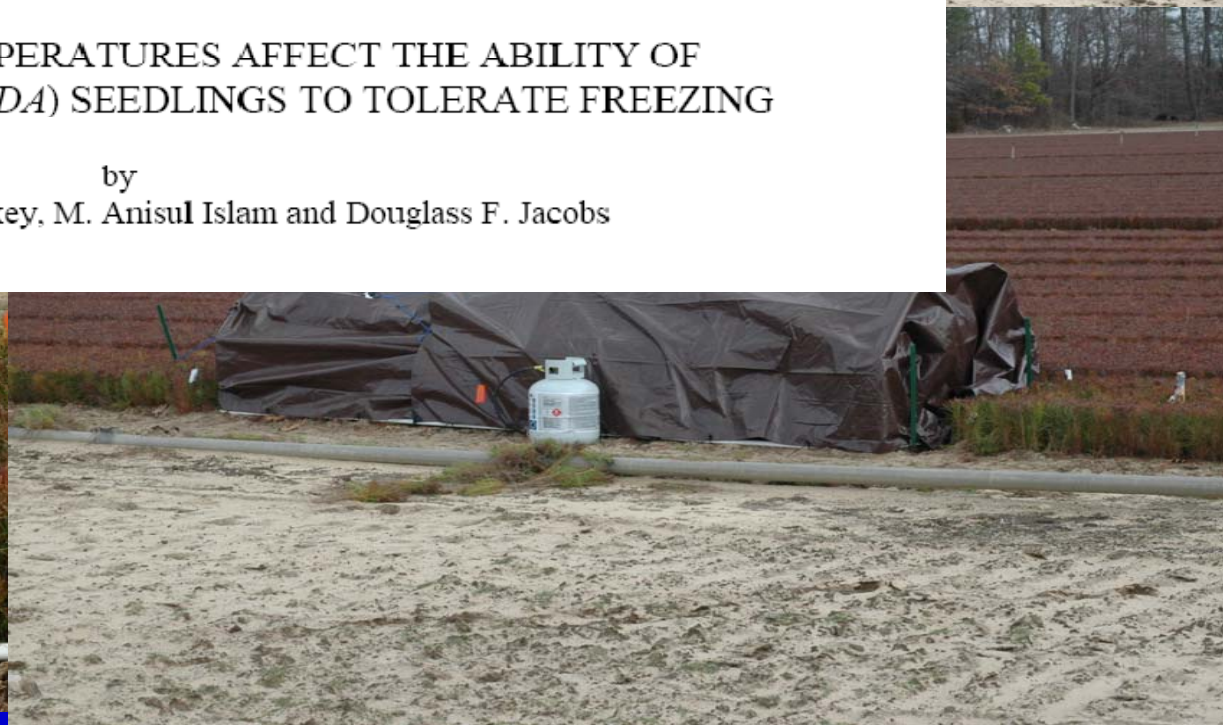


RESEARCH REPORT 08-02

WARM NIGHTTIME TEMPERATURES AFFECT THE ABILITY OF LOBLOLLY PINE (*PINUS TAEDA*) SEEDLINGS TO TOLERATE FREEZING

by
David B. South, Tom Starkey, M. Anisul Islam and Douglass F. Jacobs





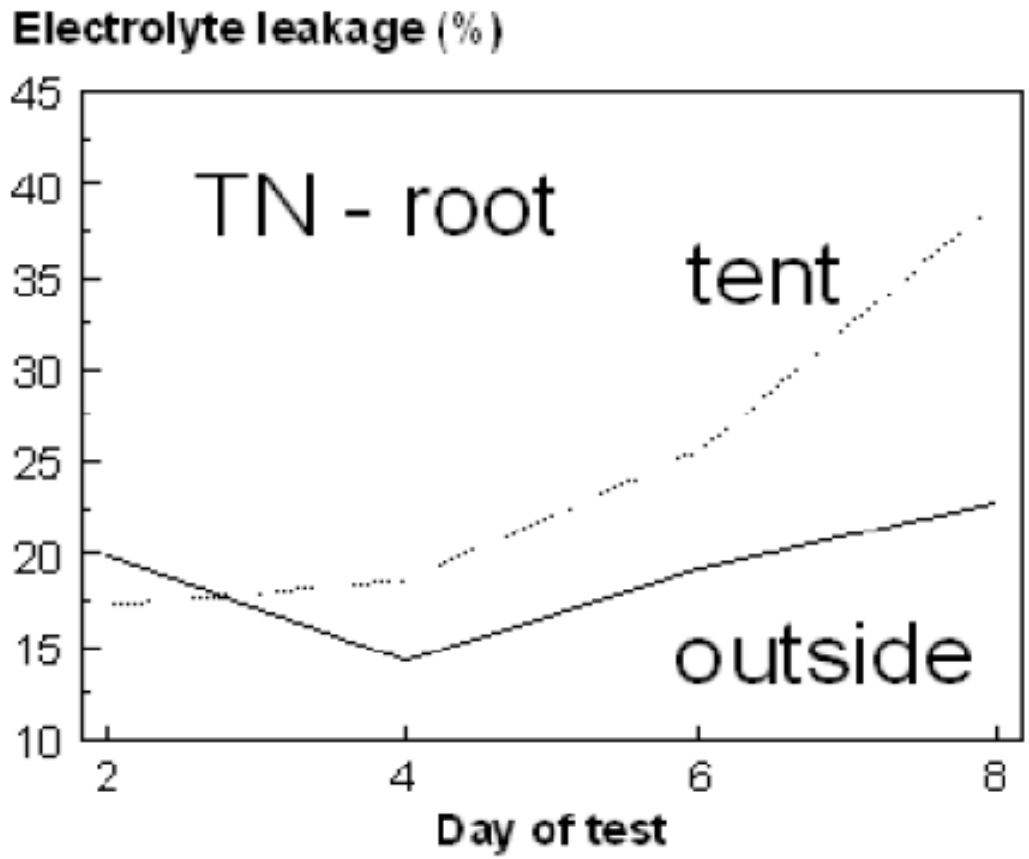
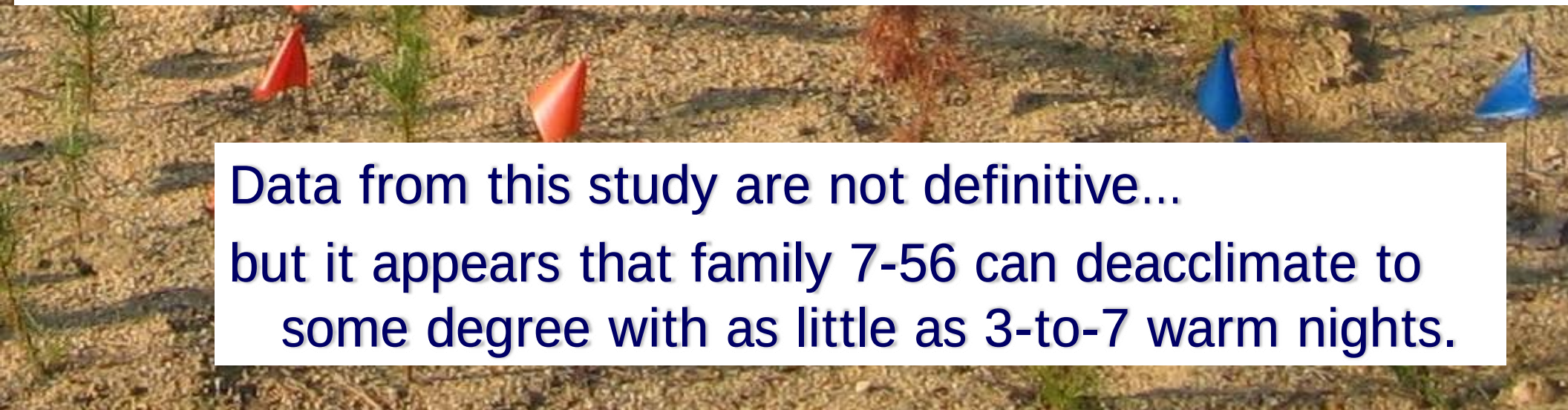

Electrolyte leakage (%)
45
40
35
30
25
20
15
10
TN - root
tent
outside
2
4
6
8
Day of test

Table 2. Effect of nighttime heat treatment (8 days) on seedling mortality due to artificial freezing (mean of -5° and -10°C) and electrolyte leakage (mean of -8° and -11°C).

		Electrolyte leakage	
Location	Seedling mortality	Roots	shoots
TN-outside	17%	22.7%	9.0%
TN-tent	29%	39.0%	6.0%
VA-outside	11%	15.2%	8.3%
VA-tent	34 %	15.3%	11.3%



Data from this study are not definitive...

but it appears that family 7-56 can deacclimate to some degree with as little as 3-to-7 warm nights.

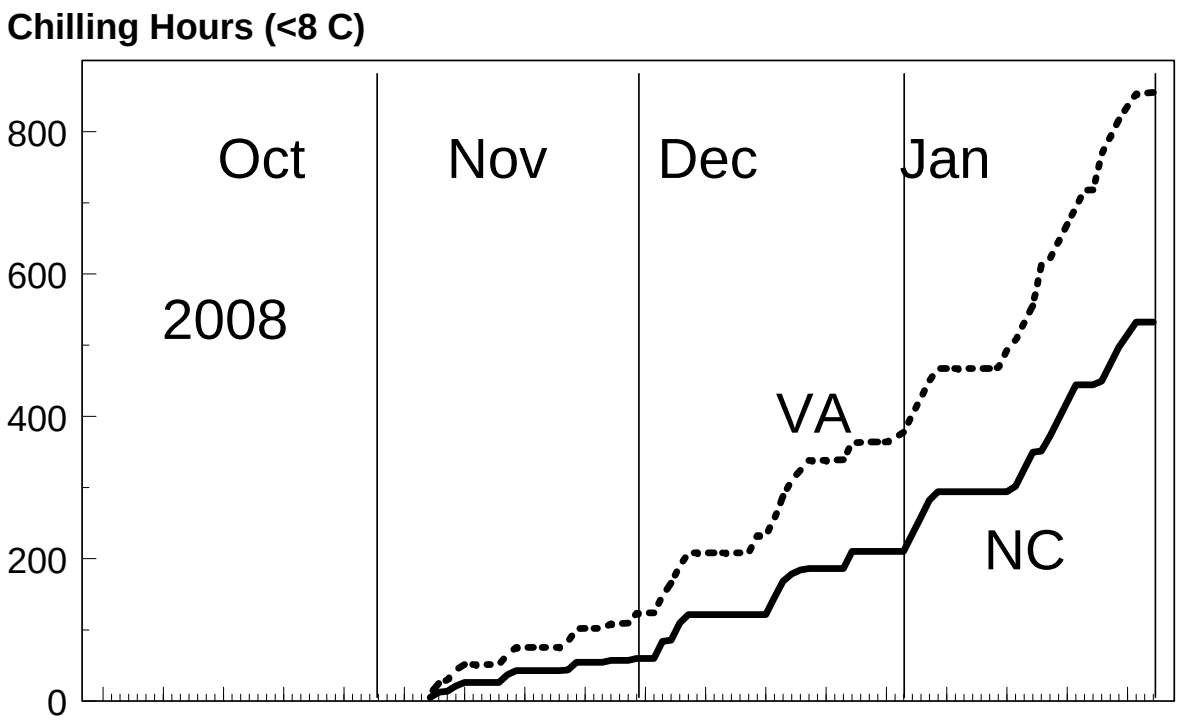
Deacclimation study 2008
VA and NC

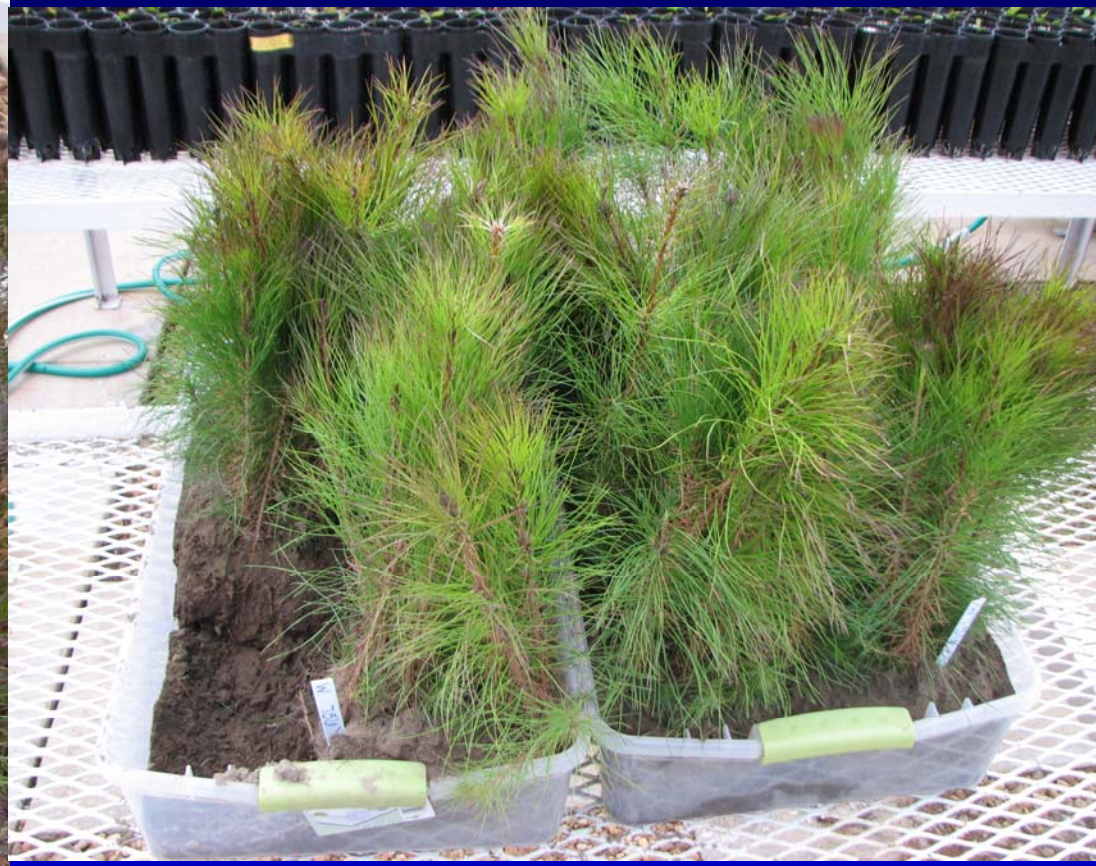
Nursery Technology Cooperative

3 5 7 days

Nursery

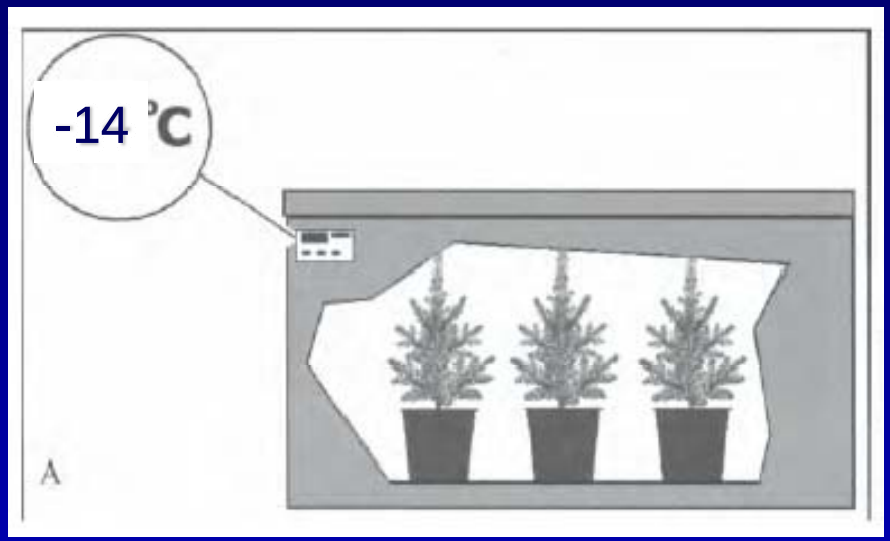
Seedlings lifted on Jan 24-25, 2008



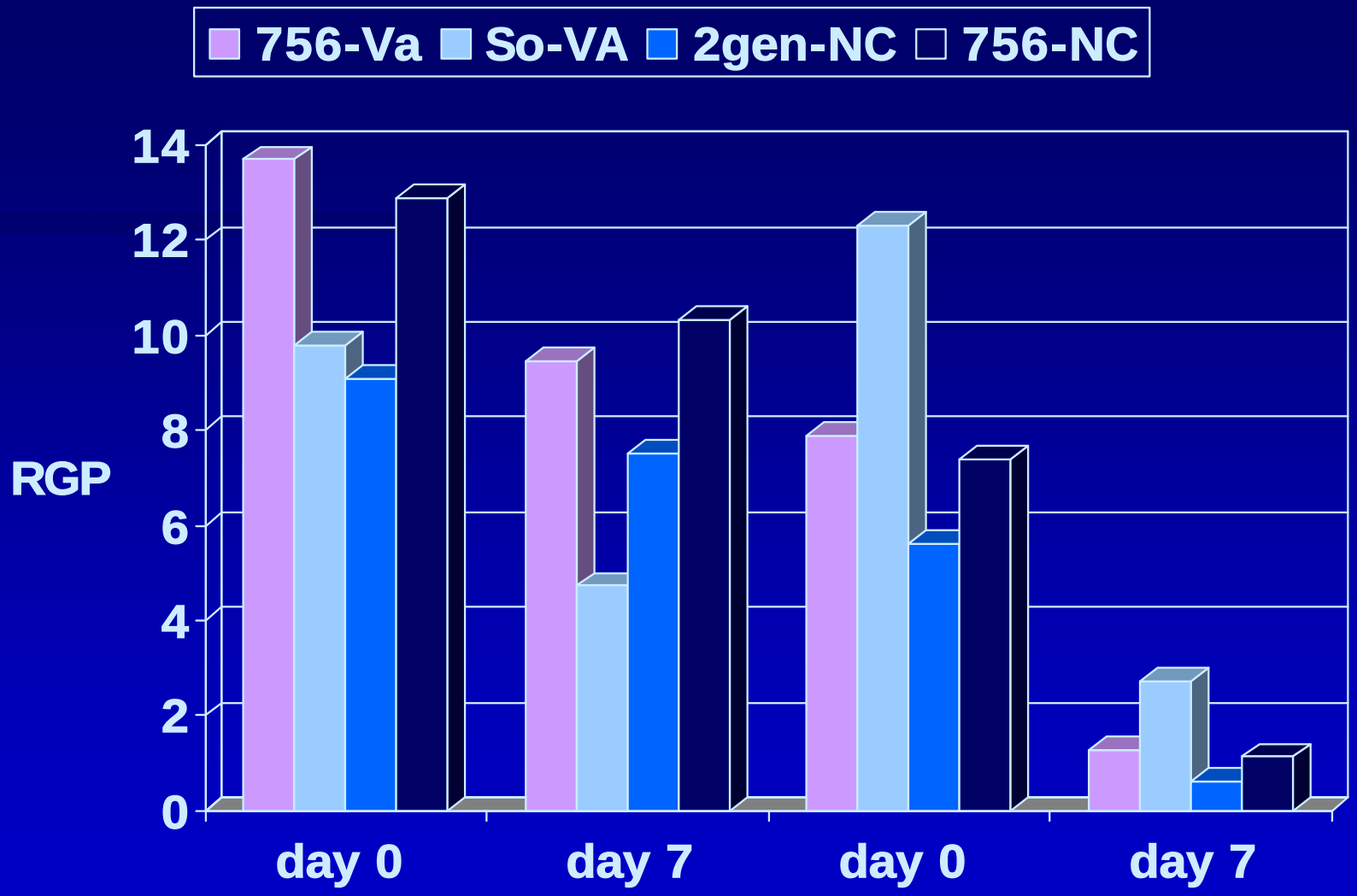


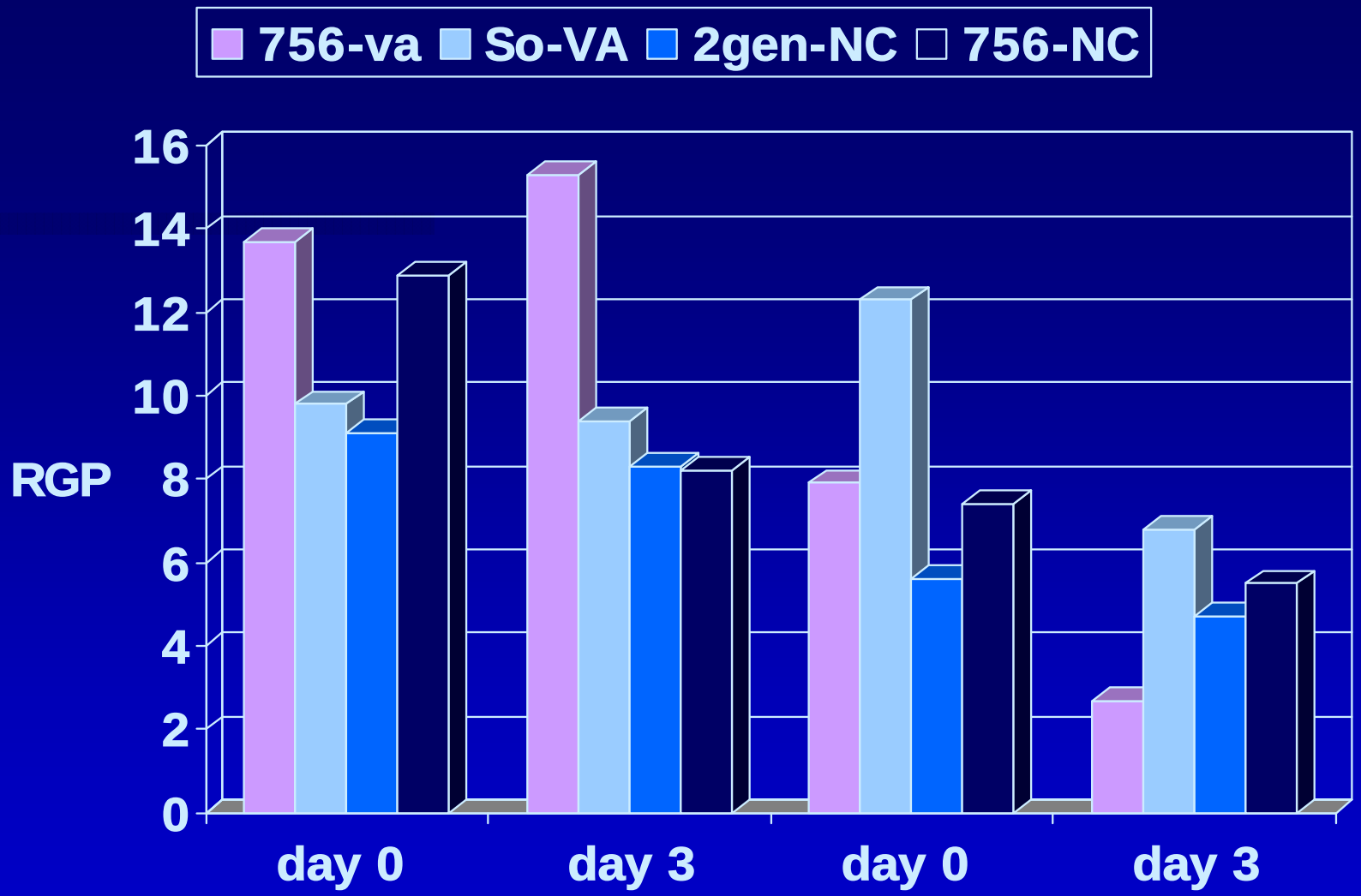
- 3, 5 or 7 days/nights

Nursery Technology Cooperative

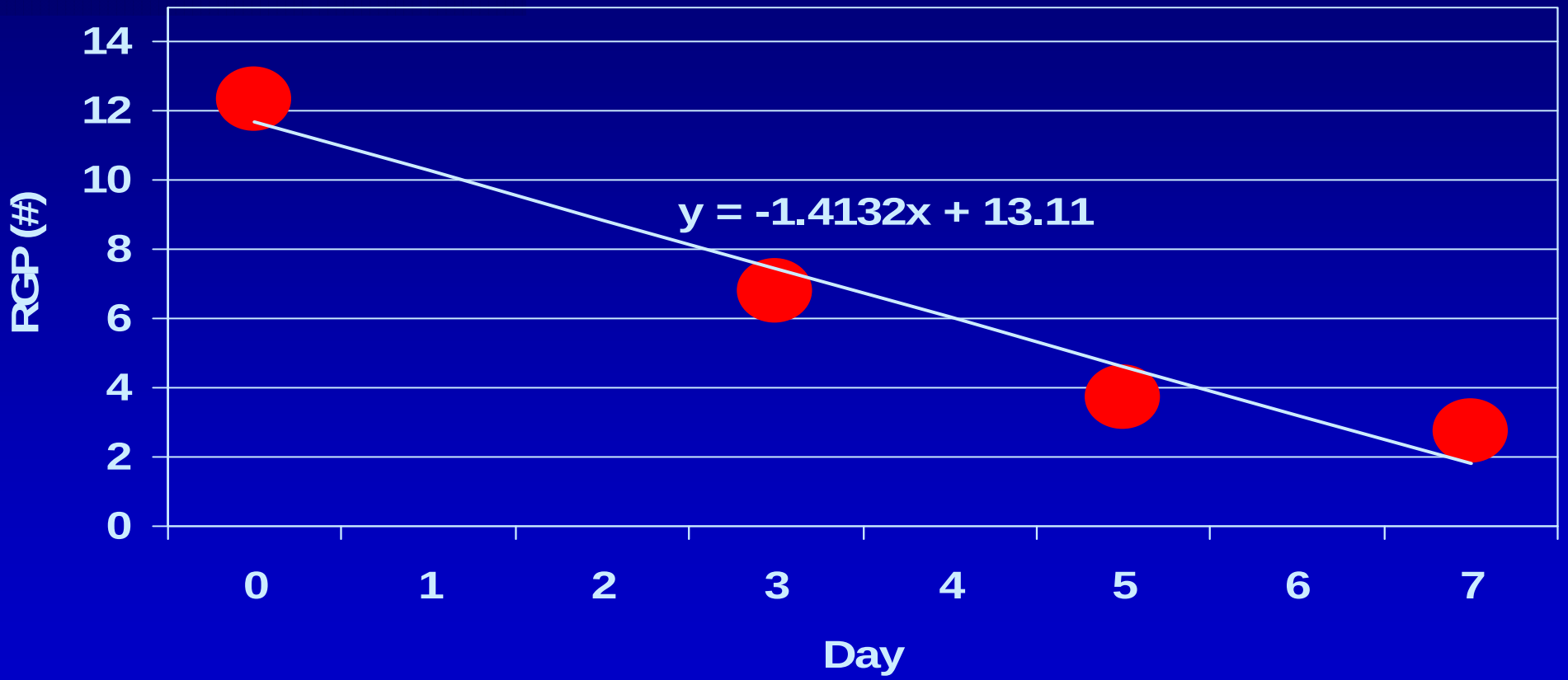


RGP and Freezing tests at OSU


756-Va
So-VA
2gen-NC
756-NC
RGP
14
12
10
8
6
4
2
0
day 0
day 7
day 0
day 7
NO FREEZE
FREEZE (7F)

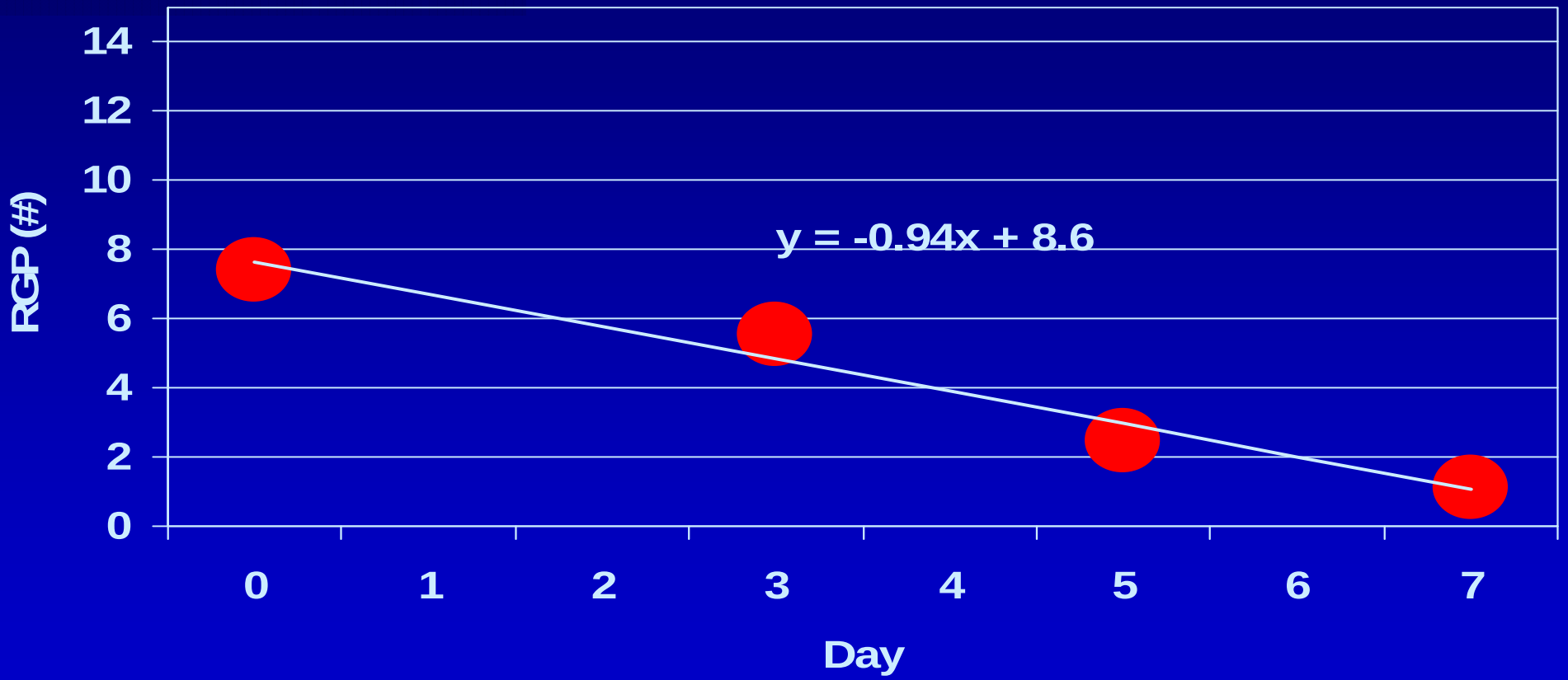

756-va
So-VA
2gen-NC
756-NC
RGP
16
14
12
10
8
6
4
2
0
day 0
day 3
day 0
day 3
NO FREEZE
FREEZE (7F)

RGP for so1997 in VA (7 F)



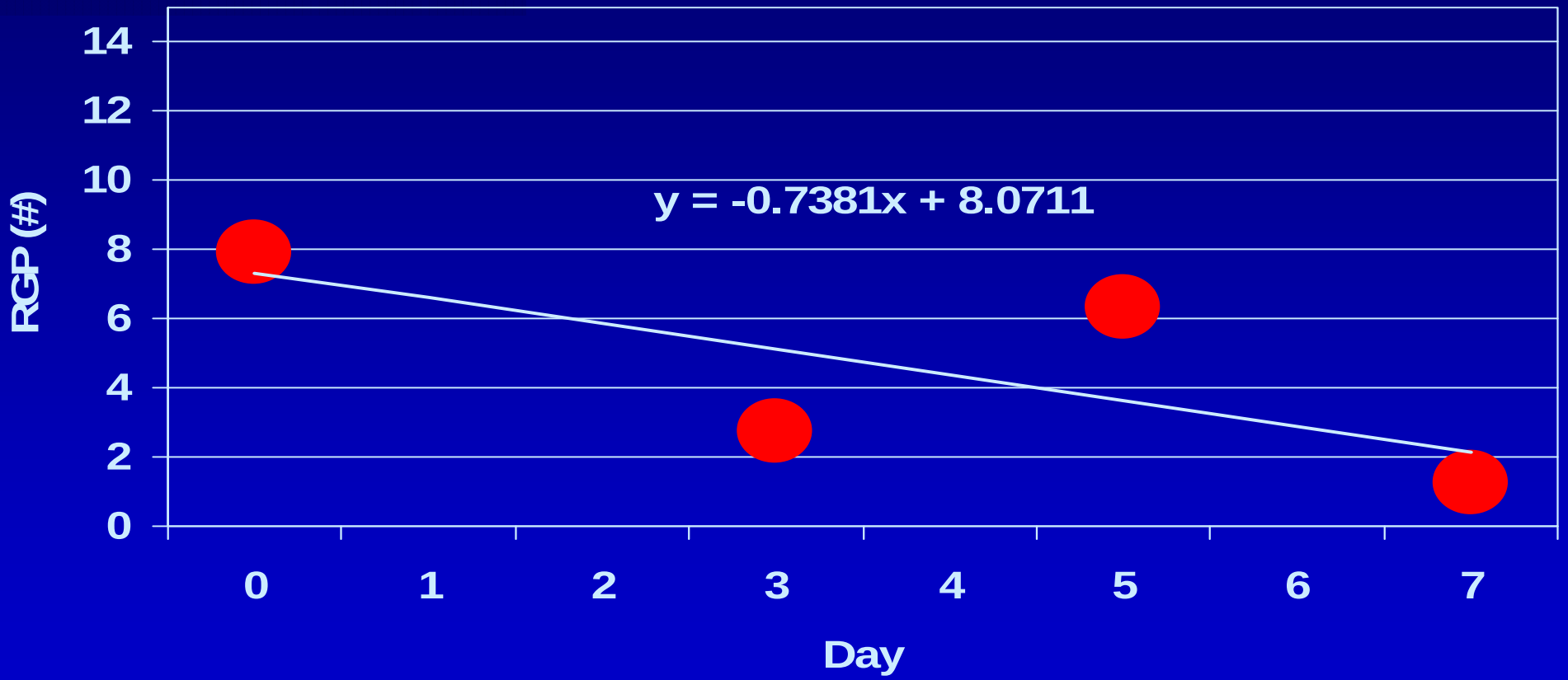
Warm days and nights

RGP for 7-56 in NC (7 F)



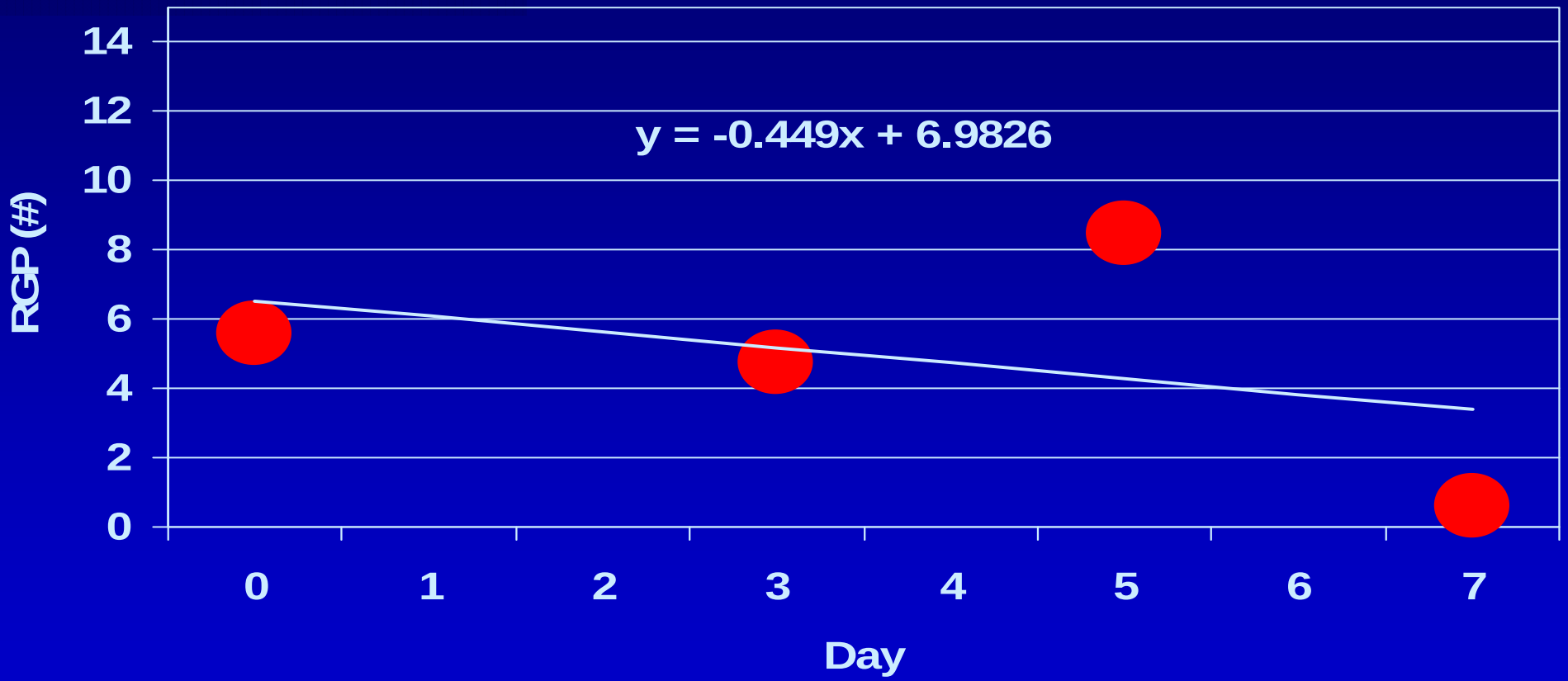
Warm days and nights

RGP for 7-56 in VA (7 F)



Warm days and nights

RGP for 2nd gen in NC (7 F)



Warm days and nights

Conclusion

- Sending seedlings to Auburn and placing them in a greenhouse reduces tolerance to freezing (as measured by RGP)

- Our greenhouse treatment reduced RGP (after freeze) by 0.4 to 1.4 new roots per warm day/night.

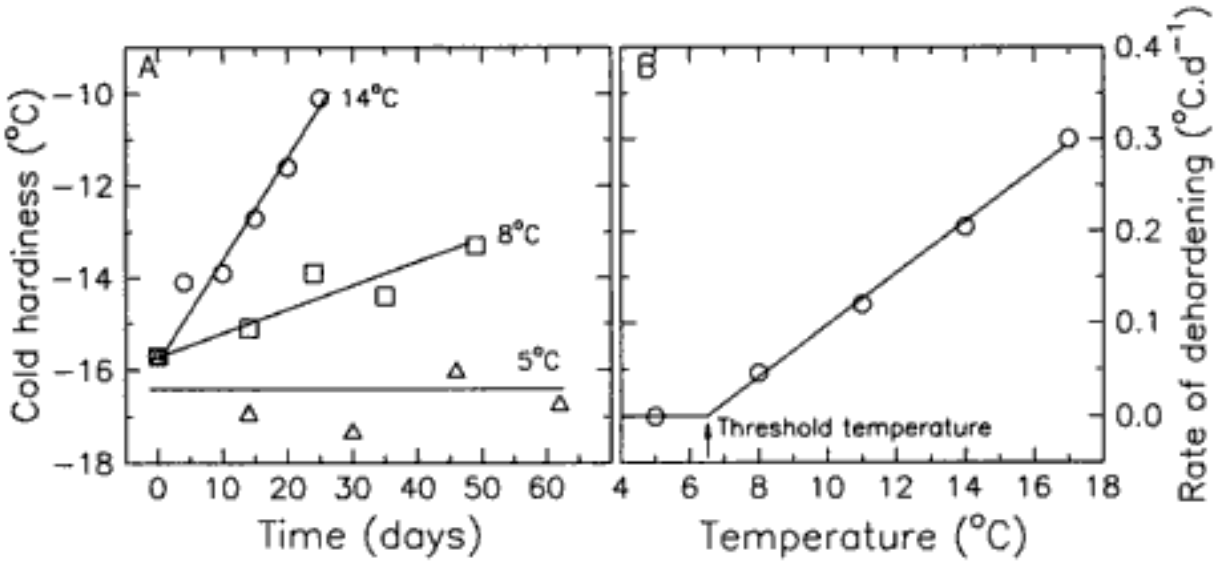
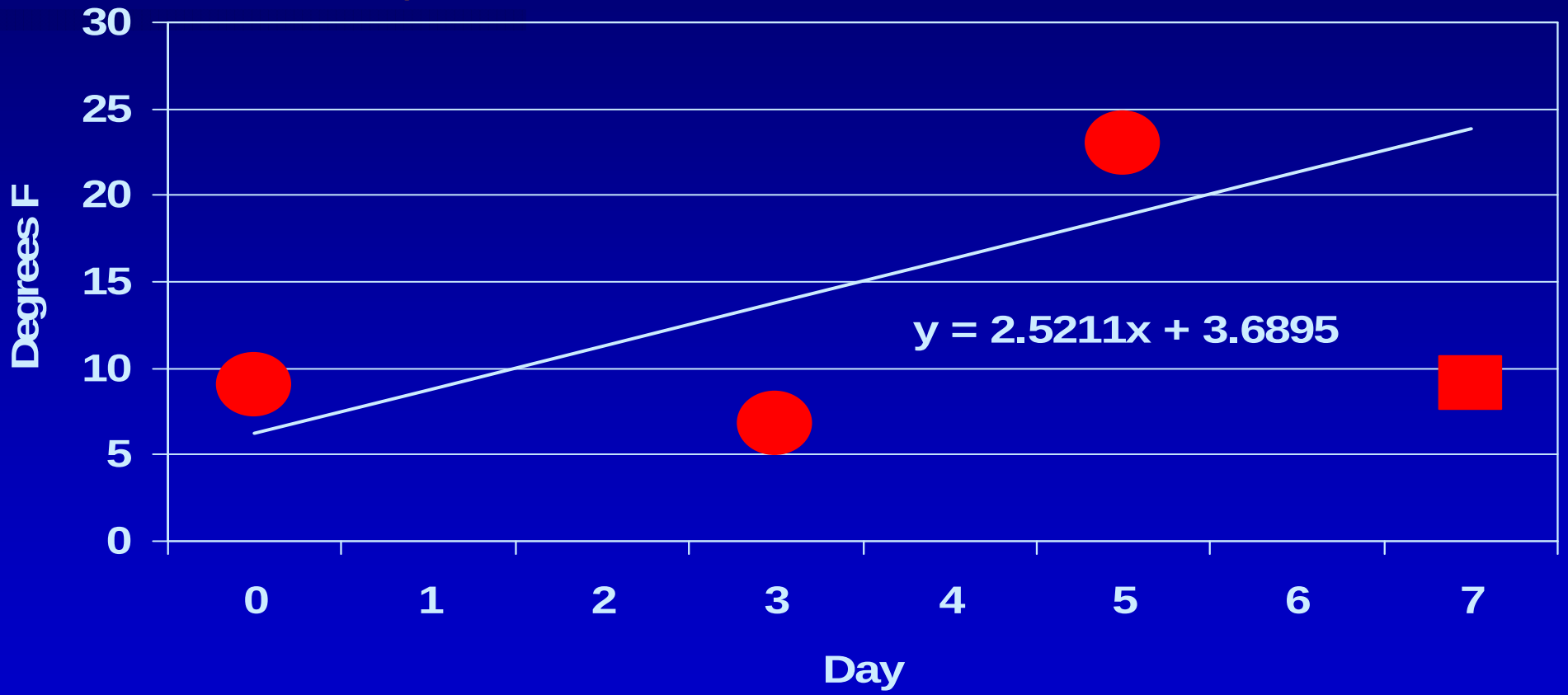


Figure 9. Temperature (A) induced losses in cold hardiness of radiata pine seedlings under 12h photoperiod and (B) the linear response function between the rate of dehardening and temperature for this species. (From Greer and Stanley 1985).

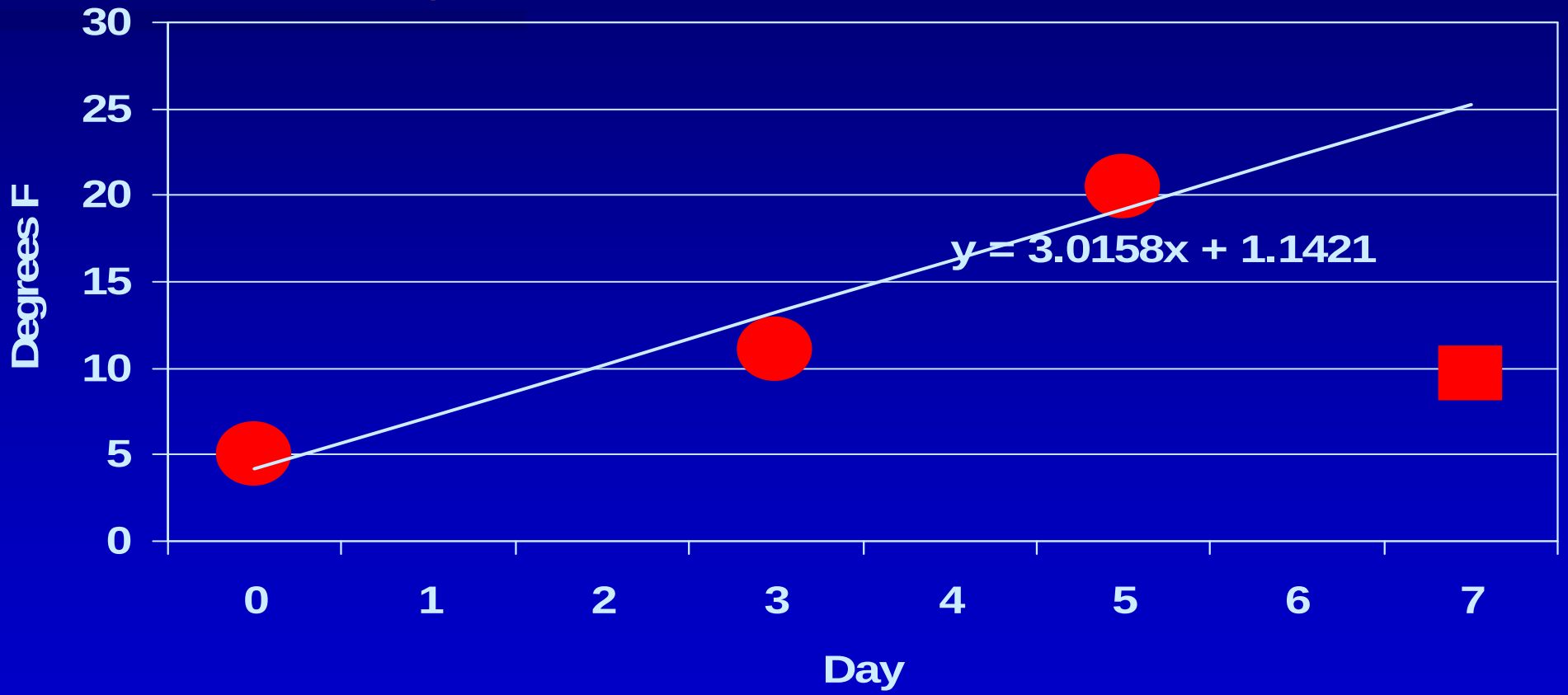
- 0.2 C per day = 0.4 F per day @ 57 F
- 0.3 C per day = 0.6 F per day @ 63 F

ELT_{10} for so1997 in VA



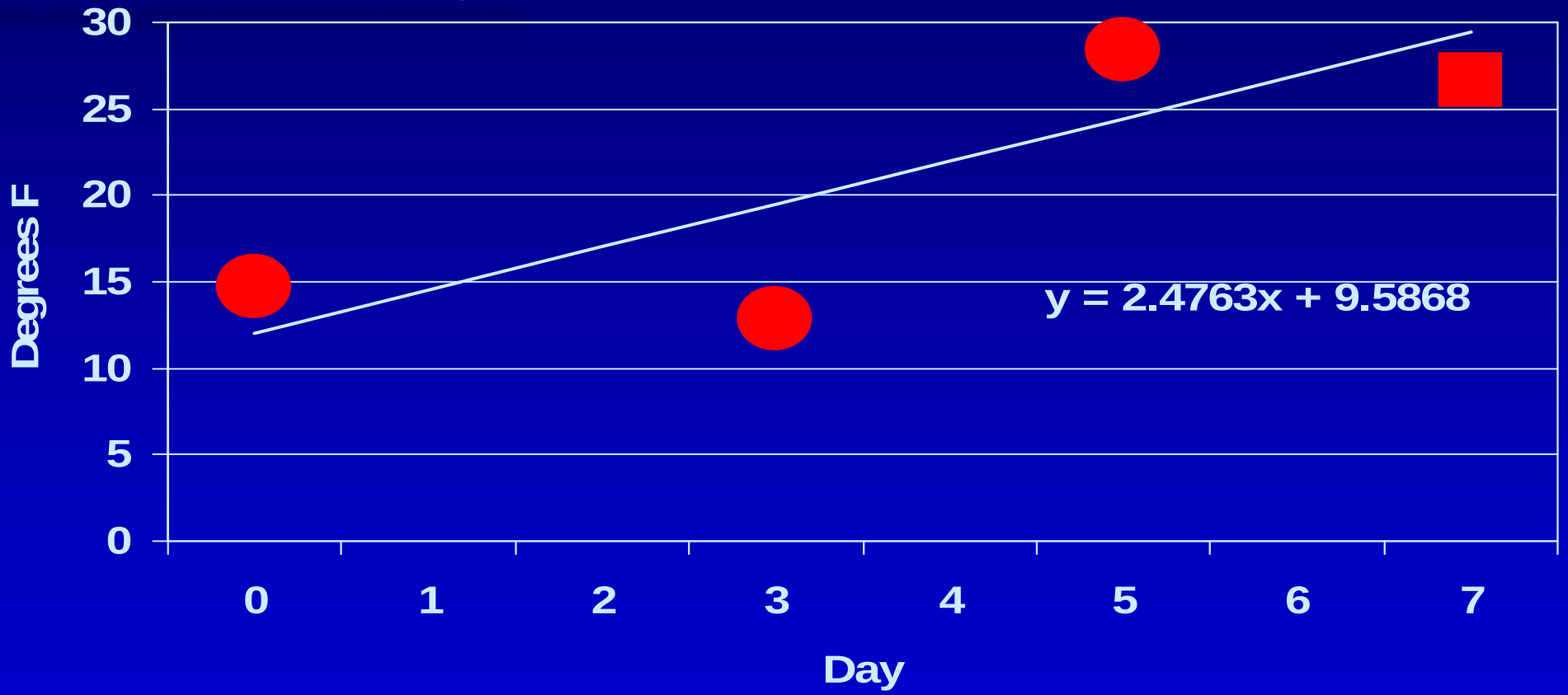
- Lost 2.5 degrees F per day @ 80 F

ELT_{10} for 2-gen in NC



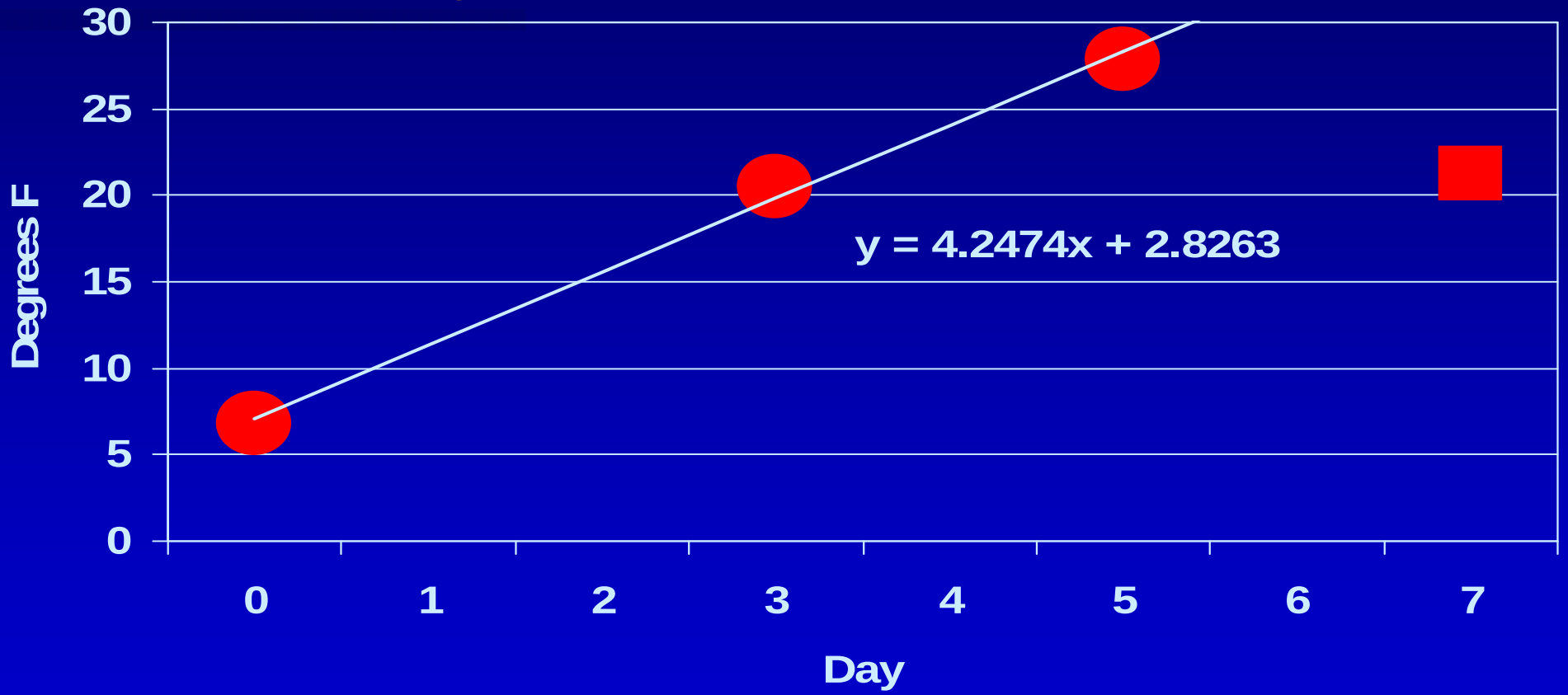
- Lost 3 degrees F per day @ 80 F

ELT_{10} for 7-56 in VA



- Lost 2.5 degrees F per day @ 80 F

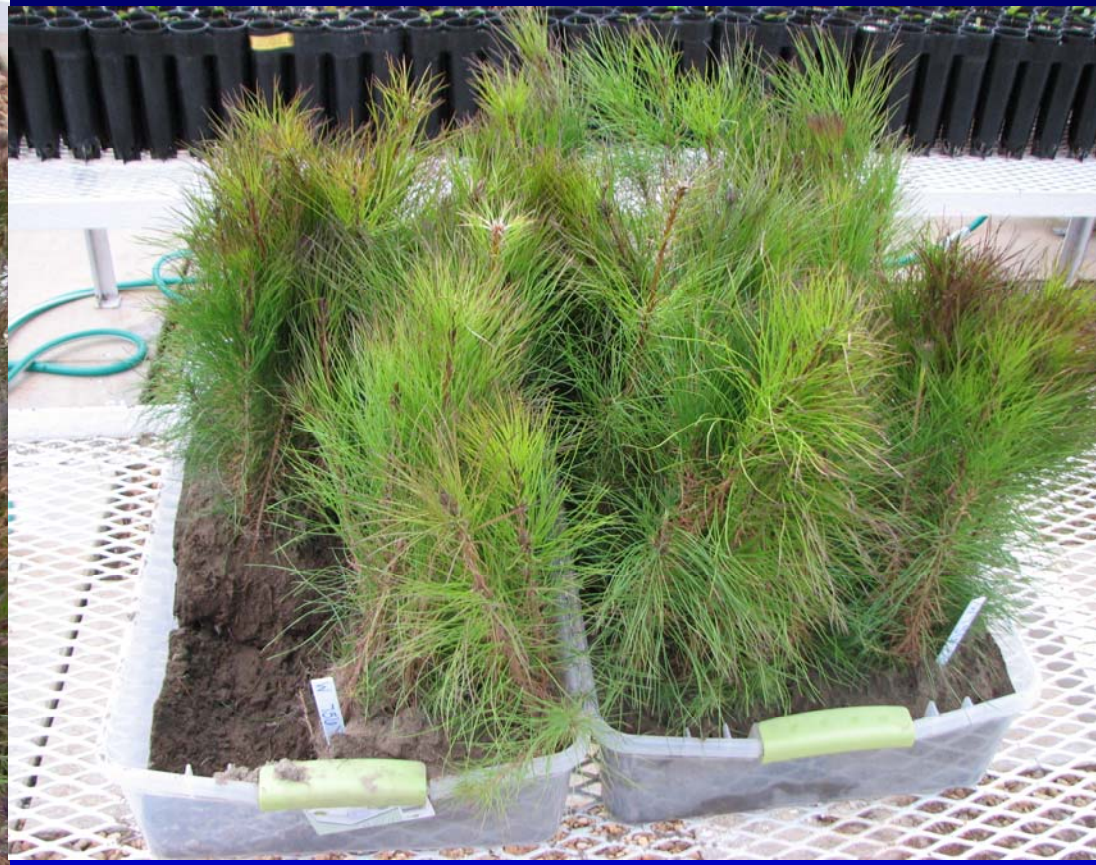
ELT_{10} for 7-56 in NC



- Lost 4.2 degrees F per day @ 80 F

Conclusion

- Under greenhouse conditions, 7-56 seedlings can become less freeze tolerant (7 to 12 F) in just 72 hours.



QUESTIONS?

Cold Nsure

David South
Tom Starkey
Dwight Stallard
Adam Howard
Auburn University
Southern Forest Nursery
Management Cooperative

info

nsure

Create certainty in fresh agro-chains

Lifting? Storage scheduling?

Take no risks, go for ColdNSure

info

nsure

Create certainty in fresh agro-chains

Pine seedlings acclimated?

Take no risks, go for ColdNSure

What is the test?

- Based on acclimation of Scots pine

Tree Physiology 26, 1297–1313

Correlating gene expression to physiological parameters and environmental conditions during cold acclimation of *Pinus sylvestris*, identification of molecular markers using cDNA microarrays

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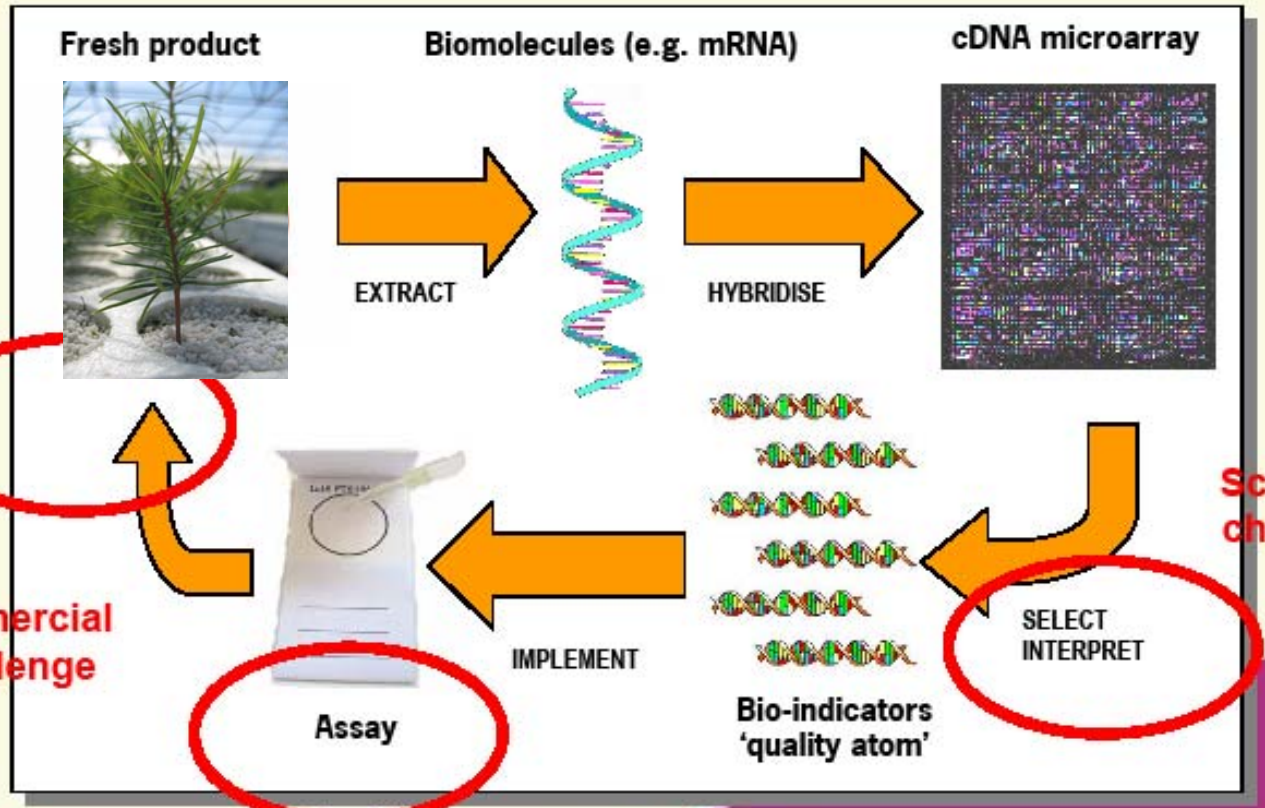
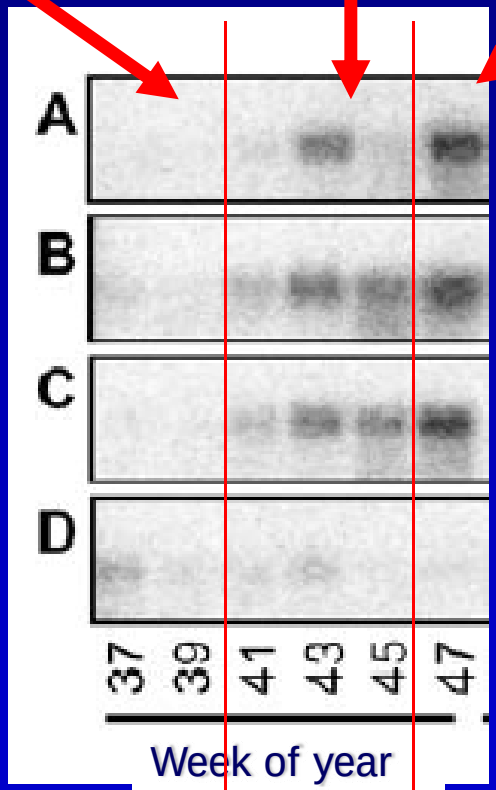
nsure

Quality atom selection



Phase 1	Phase 2	Phase 3
Not cold tolerant	Hardening has started	Hardened, ready for freezer



Phase 1	Not cold-tolerant
Phase 2	Hardening has started
Phase 3	Hardened, ready for storage

The hardiness classifications of ColdNSure

Sep-Oct

End Nov

Easy sampling

NSure assays are designed to be user friendly. The sampling kit contains all you need for easy sampling. The test cards are specially prepared in order to preserve your sample and allow for sending the card to NSure laboratory by regular mail.

The NSure diagnostic tool is broadly applicable. NSure experts are able to develop a customized quality assay, specifically targeted to your product and the quality characteristic you are most interested in. Are you interested in the possibilities for your own business, please contact us for more information.

The test is available for:

* Norway Spruce (Picea abies),
* Scots Pine (Pinus sylvestris),
* European Beech (Fagus sylvatica)
* Douglas Fir (Pseudotsuga menziesii).

A test is being developed for the Sitka Spruce. **and larch**

Monique van Wordragen

- The test can be used to:

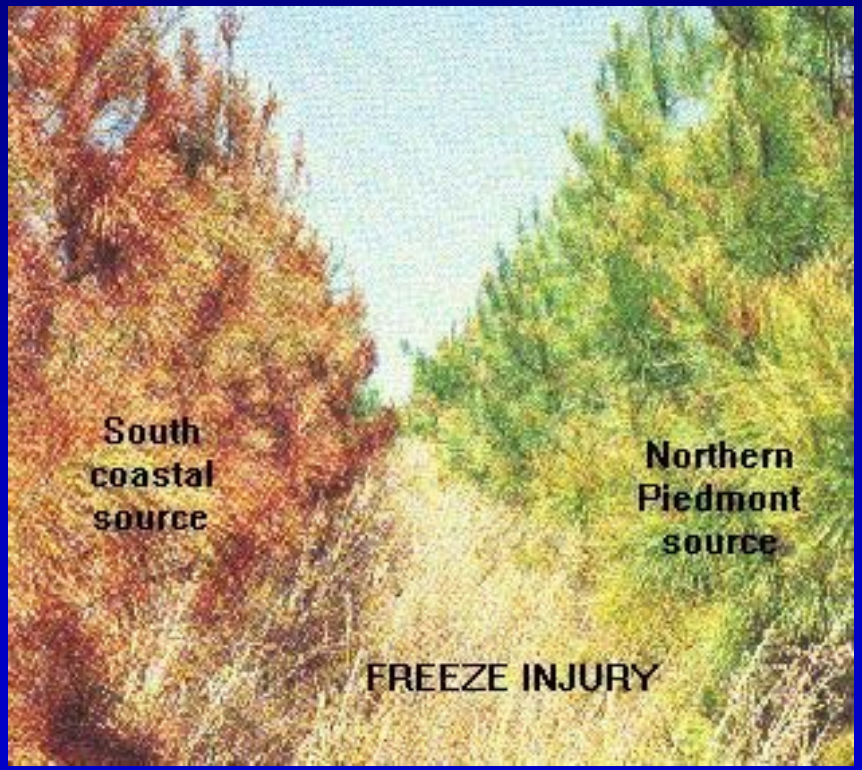
- Accurately tracking the hardening process.
- Create storage schedules by seedlot.
- Gain an early indication of the expected lifting or storage moment.

My interpretation

- Track the “freeze” hardening process.
- Create “frozen” storage schedules by seedlot.
- Gain an early indication of the expected lifting date for “frozen” storage.

Test will likely not predict when to store seedlings in a cooler (>32° F)

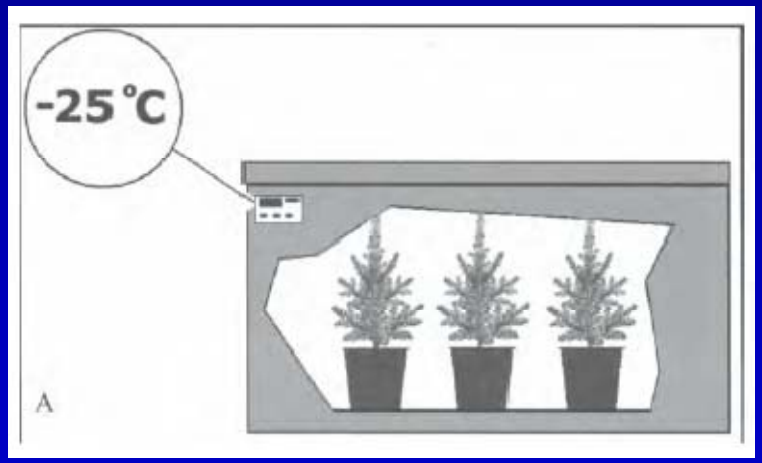
Test will likely be able to detect genetic differences

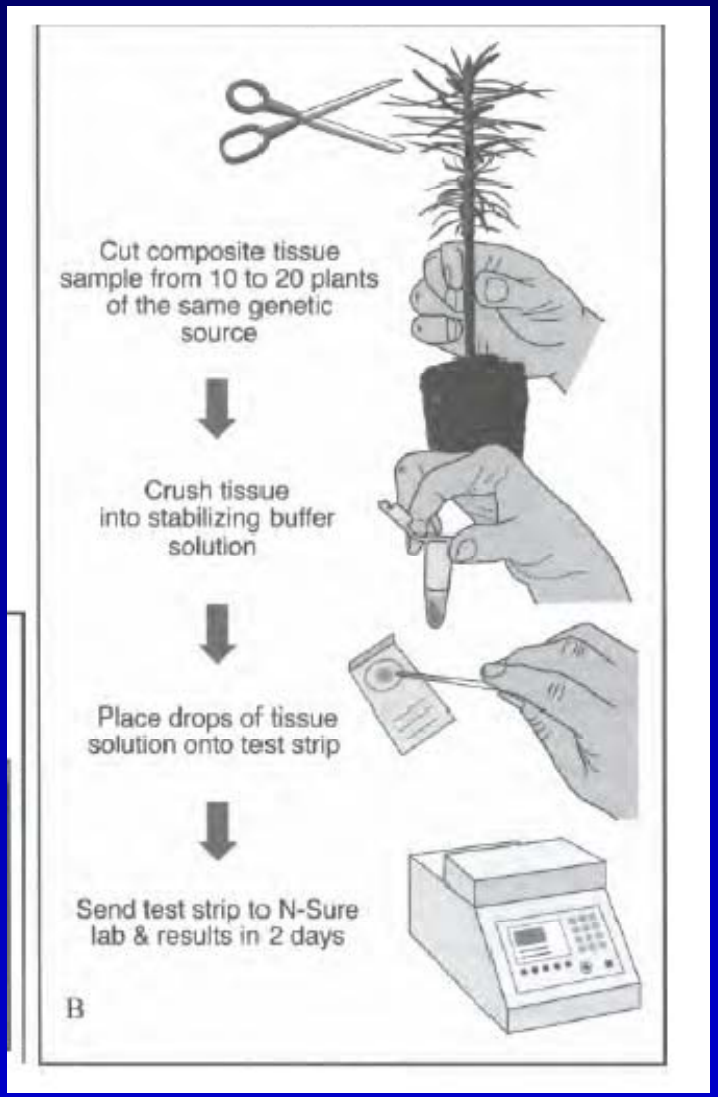


Forest Nursery Notes

Successful Trial With Innovative Cold NSure Test on Douglas fir Seedlings

Monique F. van Wordragen, Peter Balk,
and Diane Haase





News

Interest in NSure products from US and Canada results in new co-operation

During the 2006 hardening season, a trial was conducted with the Nursery Technology Cooperative at Oregon State University to determine if this test would be useful for estimating cold hardiness of Douglas-fir seedlings. Seedlings from three nurseries were sampled every two weeks from October through December. The NSure test on bud tissue showed a good relationship with results from the WPFT indicating that the gene expression method to be a possible alternative for cold hardiness testing. The correlation was consistent for seedlots from both high and low elevations, provided buds were used for the gene profiling. When needles were used, however, the correlation was poor. Analyses of the bud data distinguished three stages of cold tolerance:

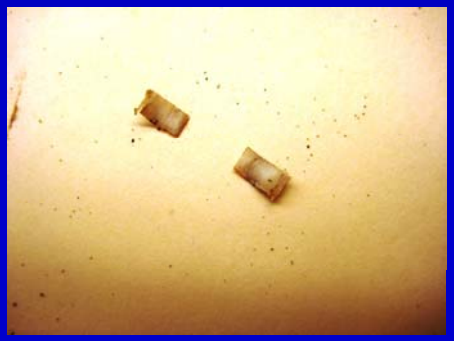
NSure phase 1 :	No frost tolerance observed
NSure phase 2 :	LT50 value between -5 and -10
	LT10 value between -1 and -5
NSure phase 3 :	LT50 value below -10
	LT10 value below -5.

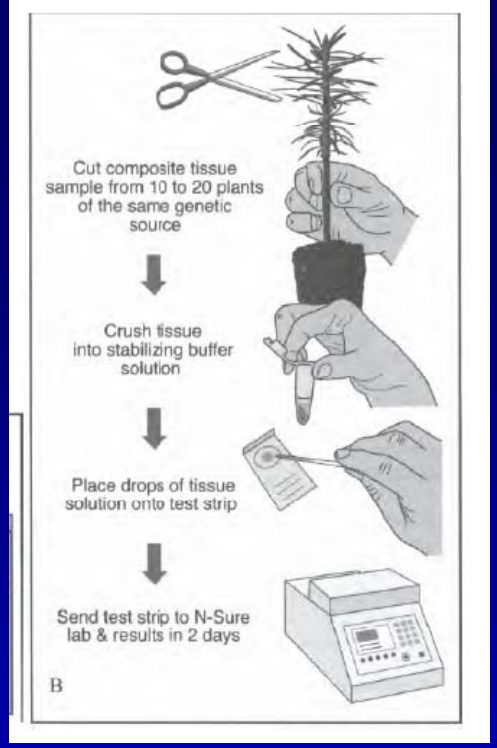
When needles were used, however, the correlation was poor. In contrast to previous findings with Norway spruce and Scots pine, this study indicates that *Pseudotsuga* needles may not be as reliable as test material.

2008 test
bareroot stock in VA
container stock in AL

Sample every 3 weeks


7-56





Stem Needle Bud

Estimated Lethal Temperature

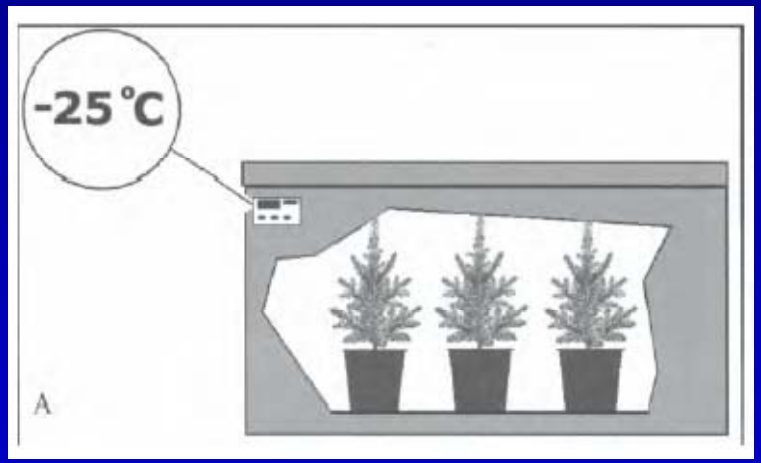
Assessment Date: December 16 Freezing Temp: -12°C

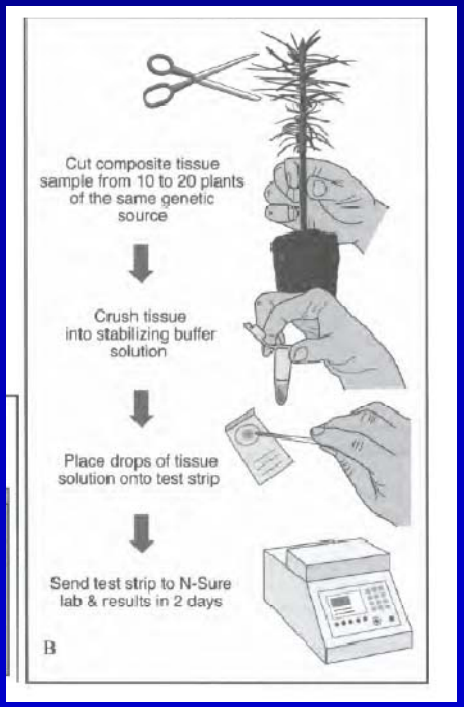
tree #	# dead buds	needle damage	Cambium damage			vitality	explanation
			bottom	middle	top		
1	4/6	45%	2	2	1	0	> 50% of buds are dead
2	1/5	"	1	1	1	1	live
14	2/6	"	0	2	0	0	bottom cambium killed
15	5/8	"	2	2	2	0	>50% bud kill
					% KILLED:		
					3 live seedlings (vitality = 1)		
					3 questionable seedlings (vitality – 2)	56.7%	
					7 dead seedlings (vitality = 0)		
					Total = 8.5 killed out of 15 total		

Key: cambium/vitality - 0=dead; 1=healthy; 2=questionable

Which method will produce a cold-tolerance curve?

The ELT method or the Cold Nsure method?





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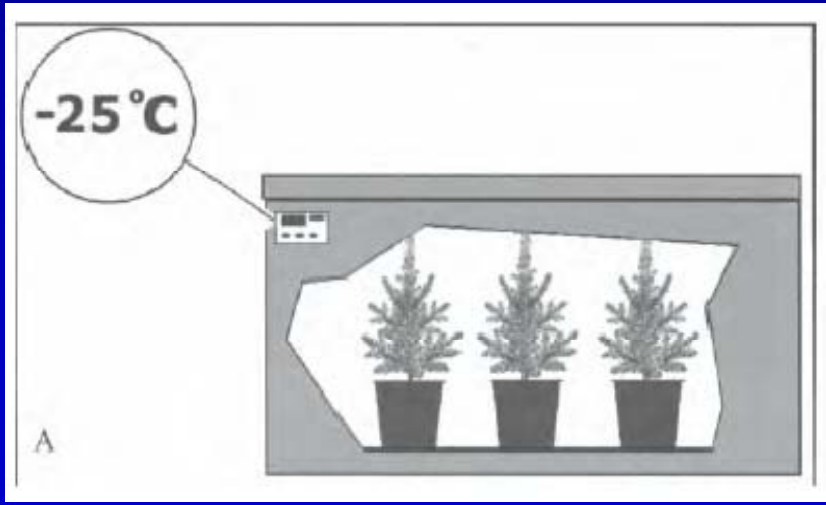
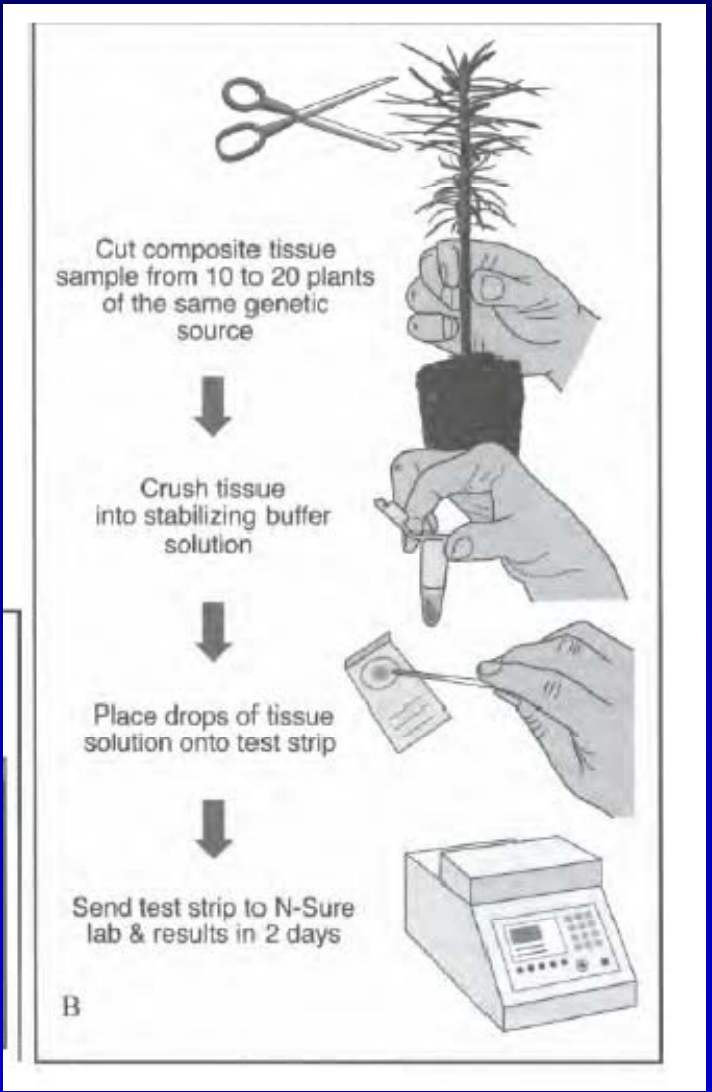
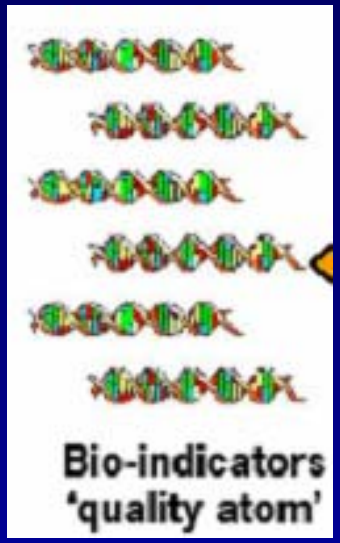




Forest trees

- ColdNSure European Beech
- ColdNSure Picea
- ColdNSure Pinus
- ColdNSure Pseudotsuga



- Eight putative indicators were selected.
- Six supposedly relate to cold stress.
- Two supposedly relate to cell division (vitality indicator)

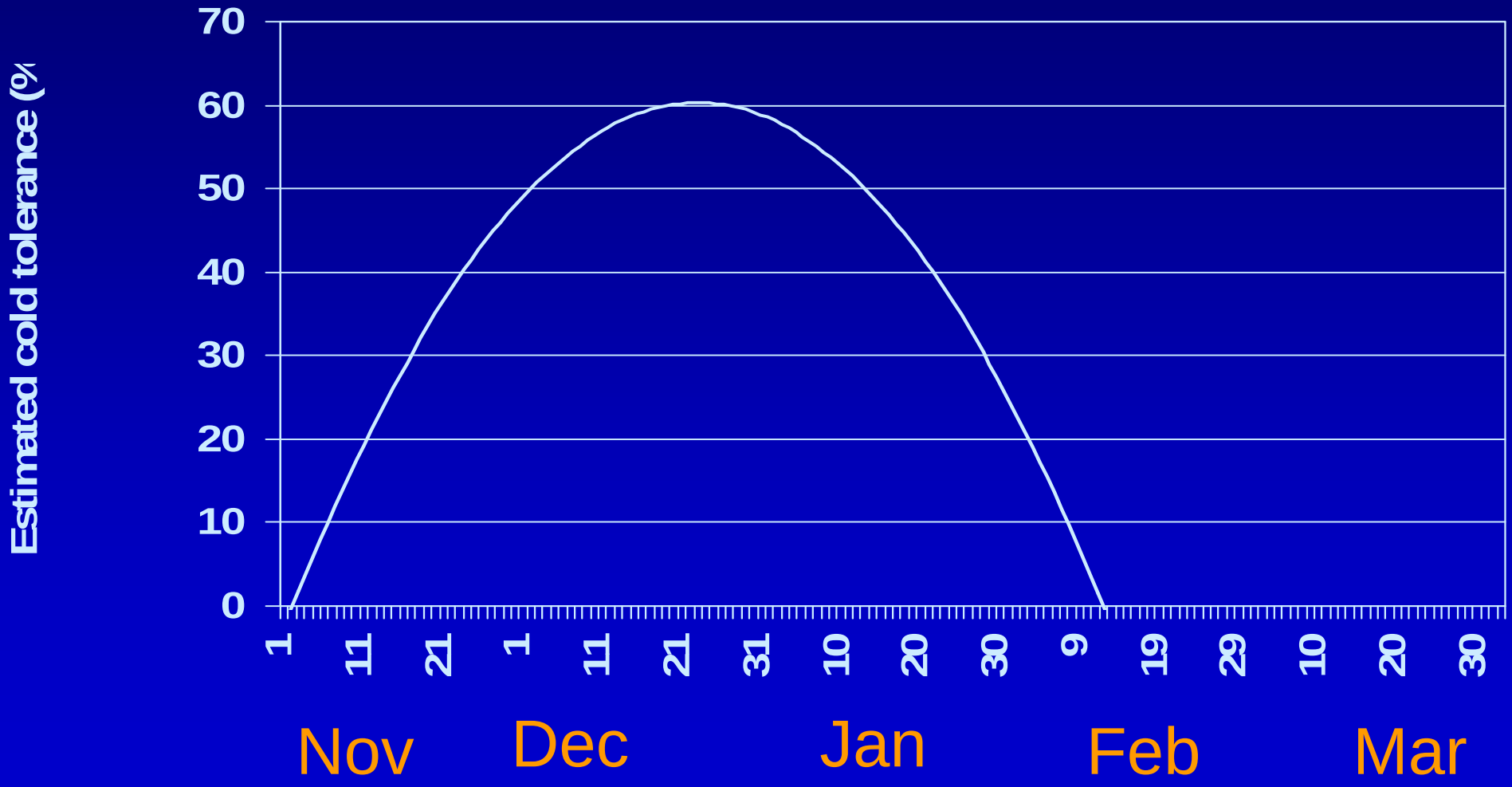
Two of the cold stress indicators (CT1 and CT2) and one vitality indicator (V1) were detectable at reasonable levels in loblolly pine bud tissue.

- The expression levels of the other indicators appeared to be to low to draw any conclusion.

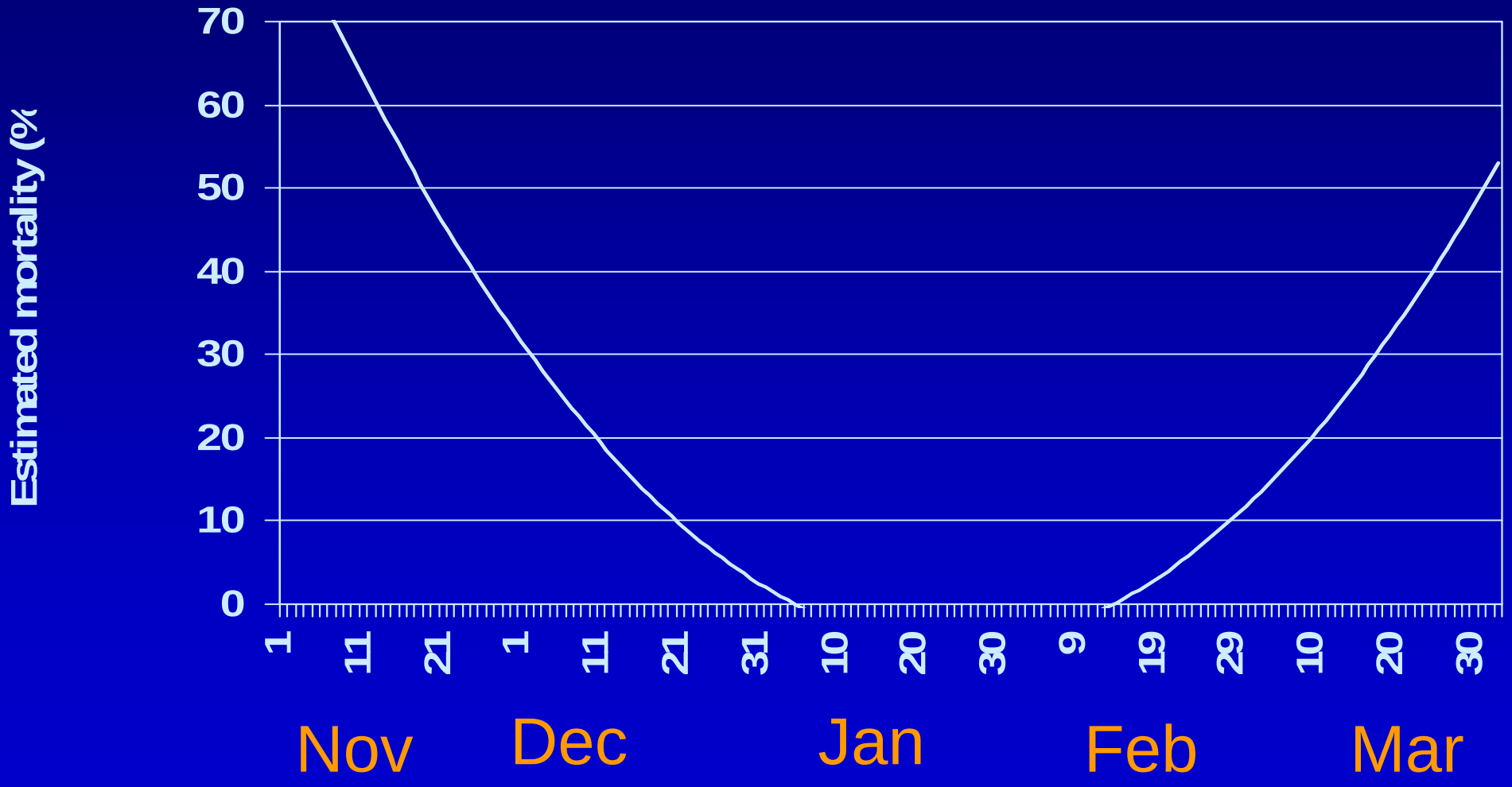
Bud... needle.. or stem?

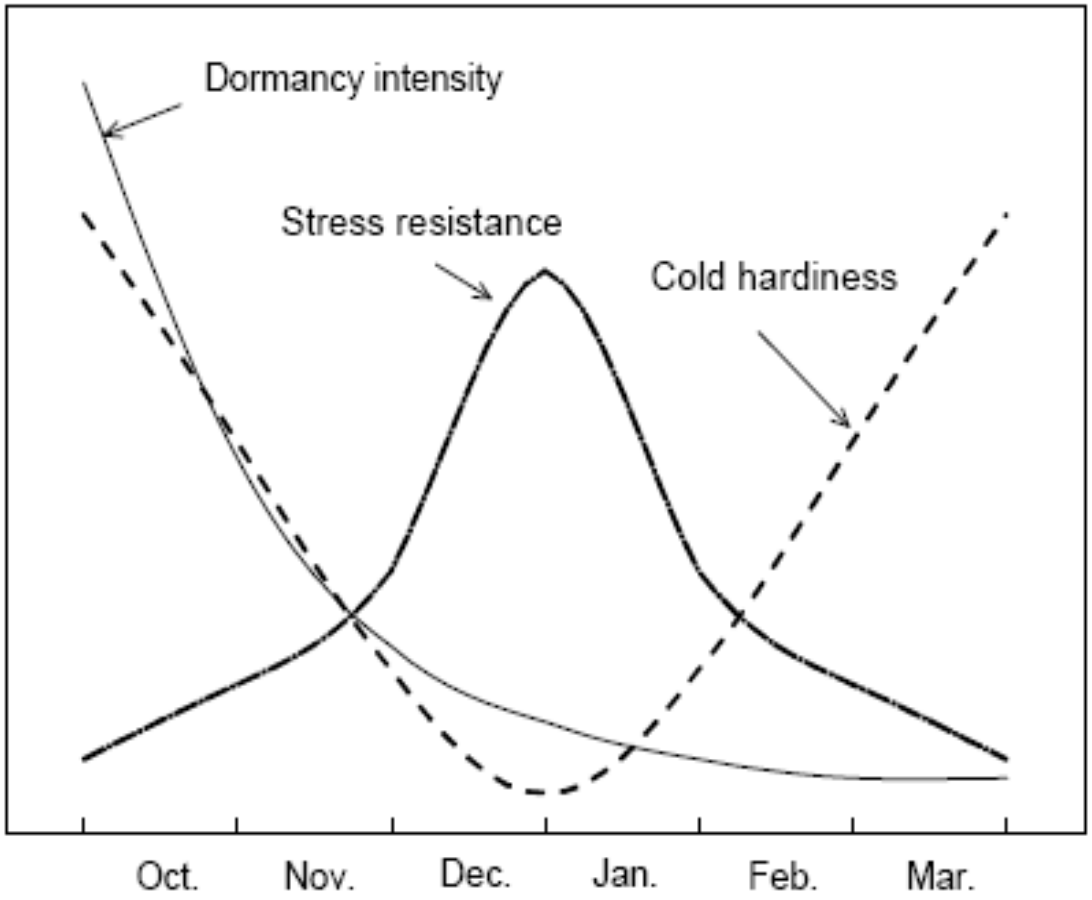
- RNA yields from the three different tissue types were evaluated by “real-time polymerase chain reaction” (RT-PCR) using primers developed for a ribosomal gene. It appeared that from buds, RNA yield was sufficient for further measurements. However RNA yields from needles and stem (at root collar) were extremely low, about 1000 to 10,000 times lower than the yield from buds.

Theoretical pattern of cold tolerance

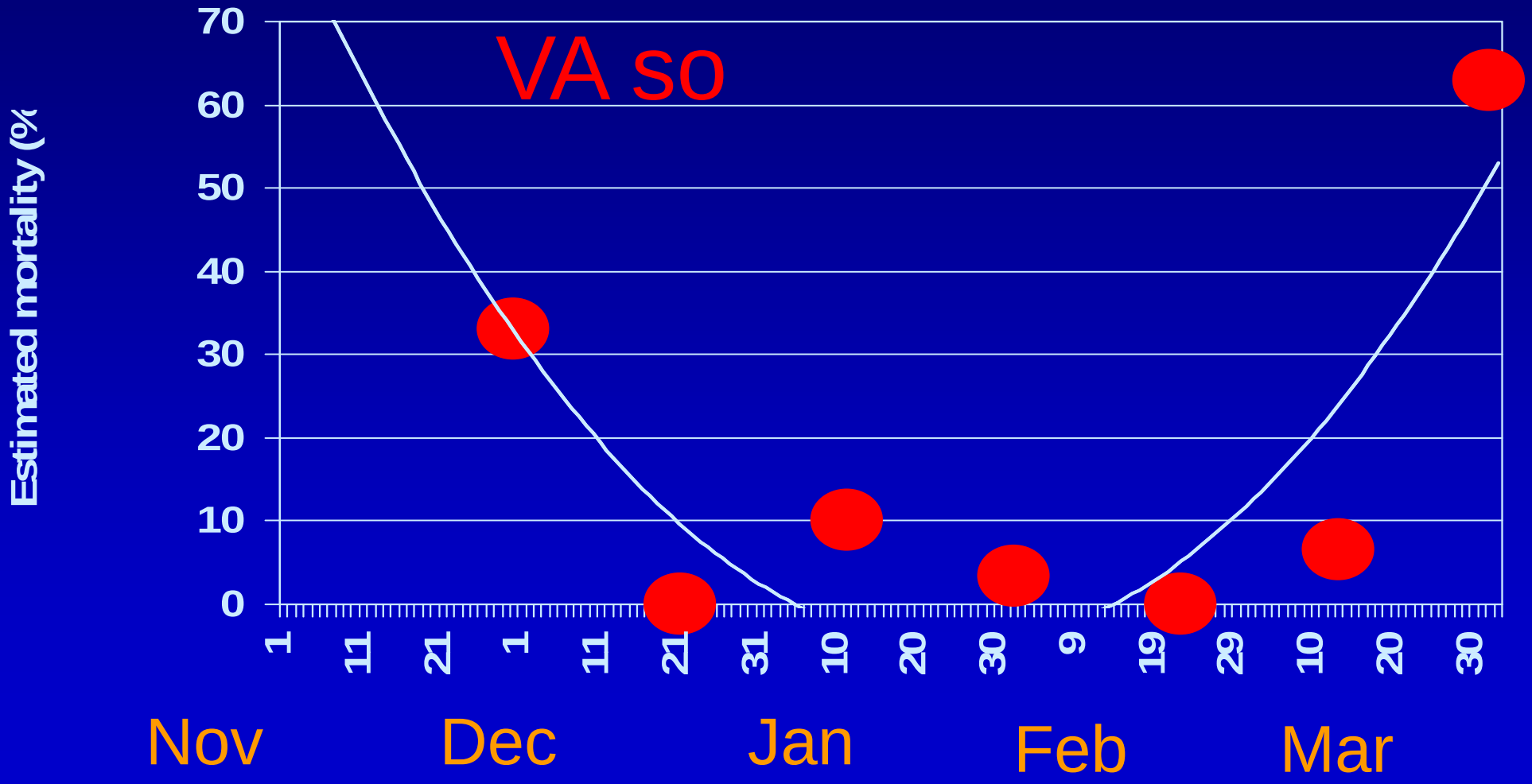


Theoretical pattern of cold tolerance

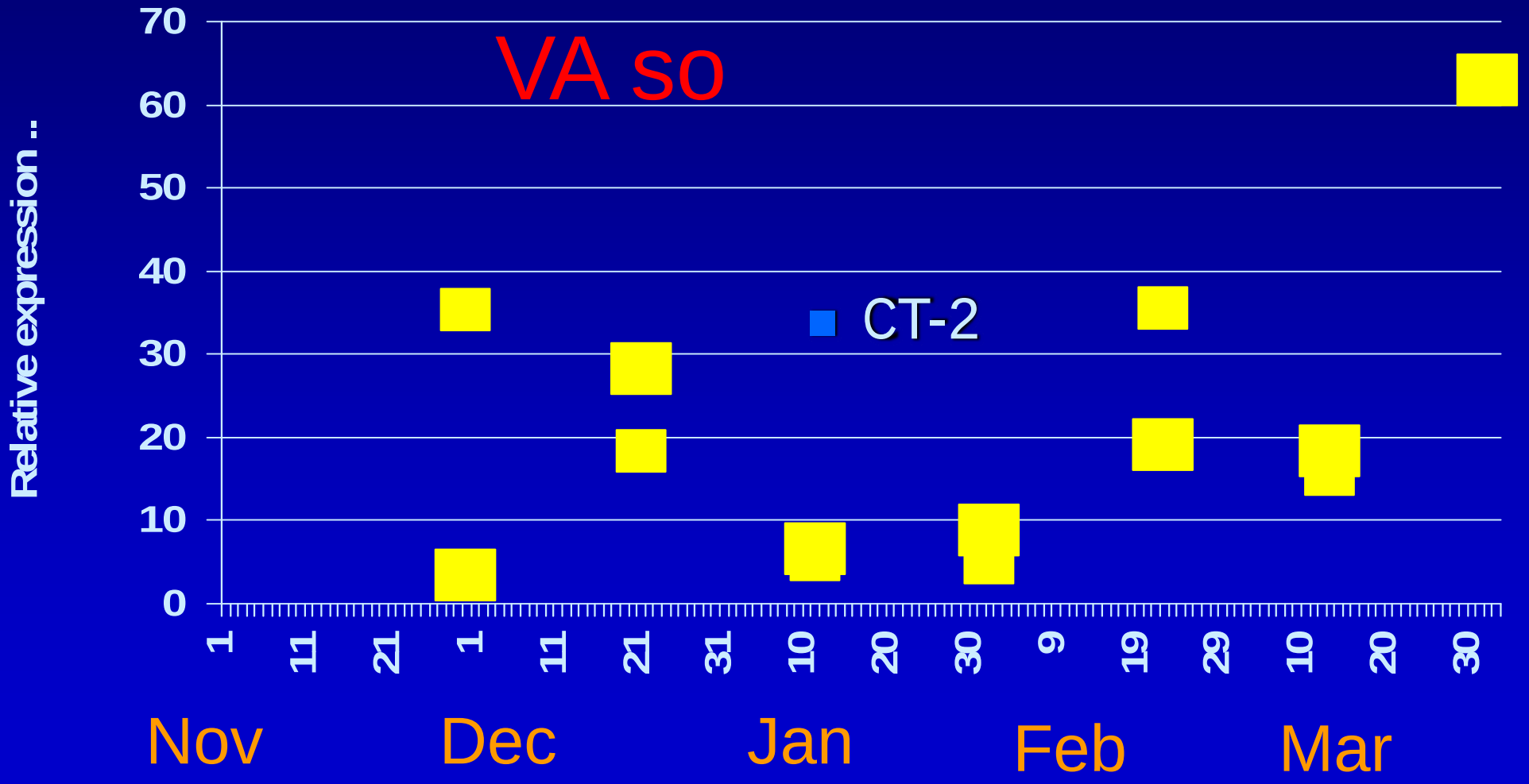



Dormancy intensity
Stress resistance
Cold hardiness
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.

Estimated mortality to -10 C freeze



Variation in coldstress indicator



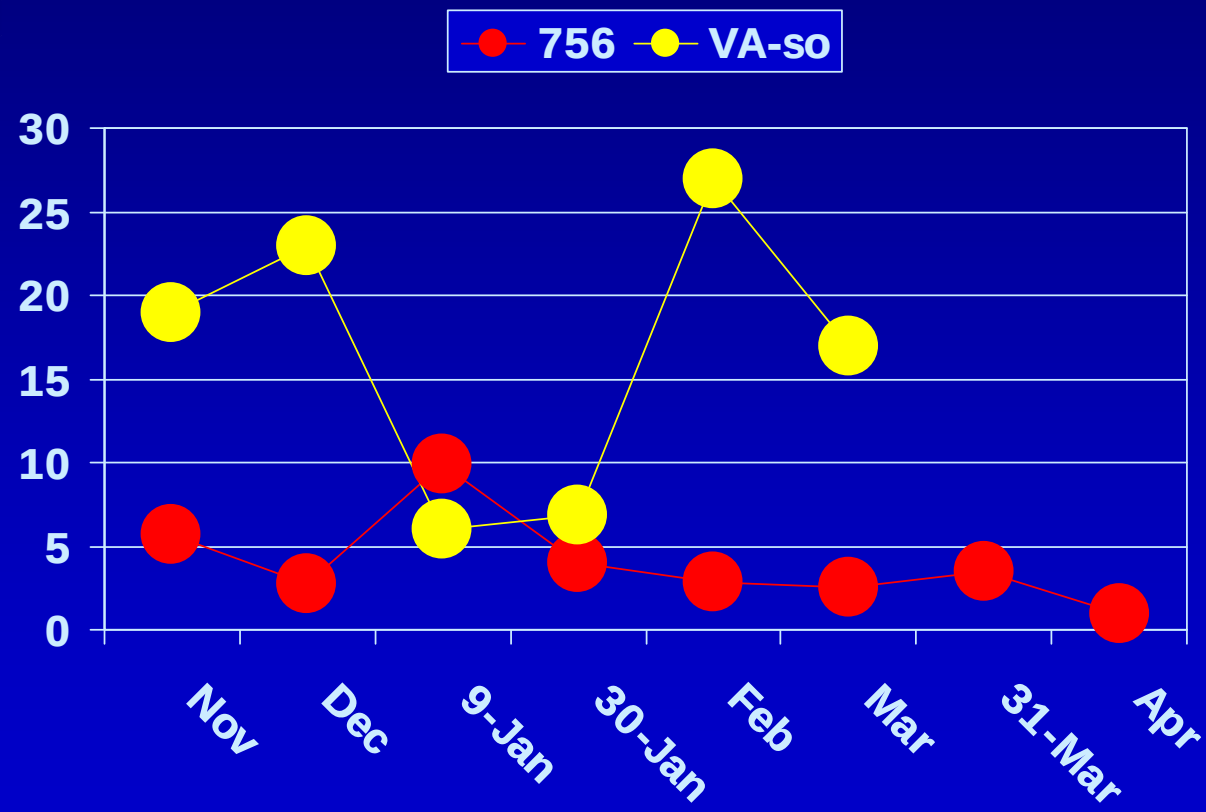
Genotypic differences

- The overall level of expression of the cold stress indicator PTC2 is relatively high in VA Piedmont with two clearly distinguishable peaks (28 November and 20 February). The situation is different for the other two sources (7-56 and AL-coastal) where the expression is low or declining.

- Test will likely be able to detect genetic differences

CT-2

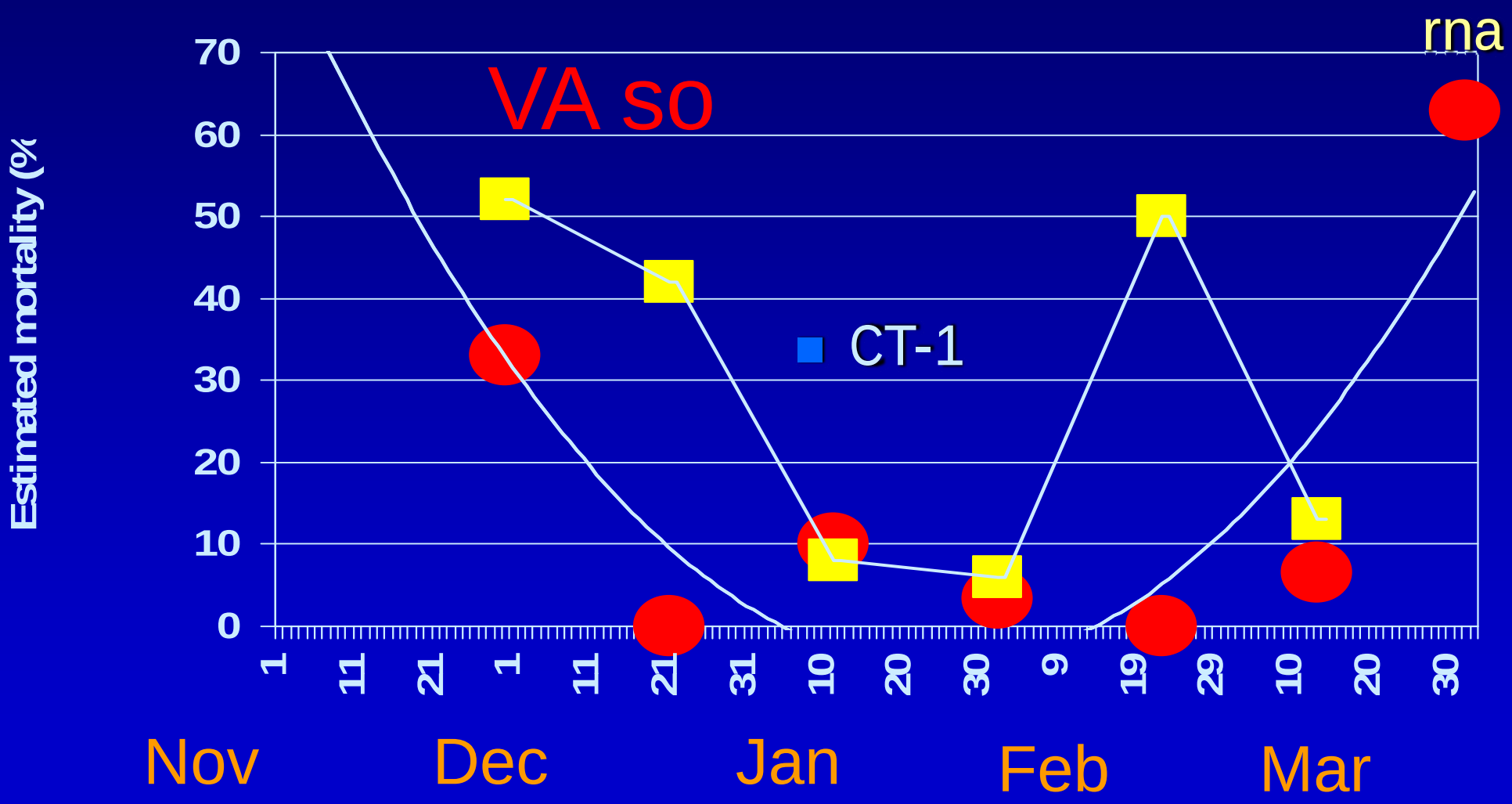
rna



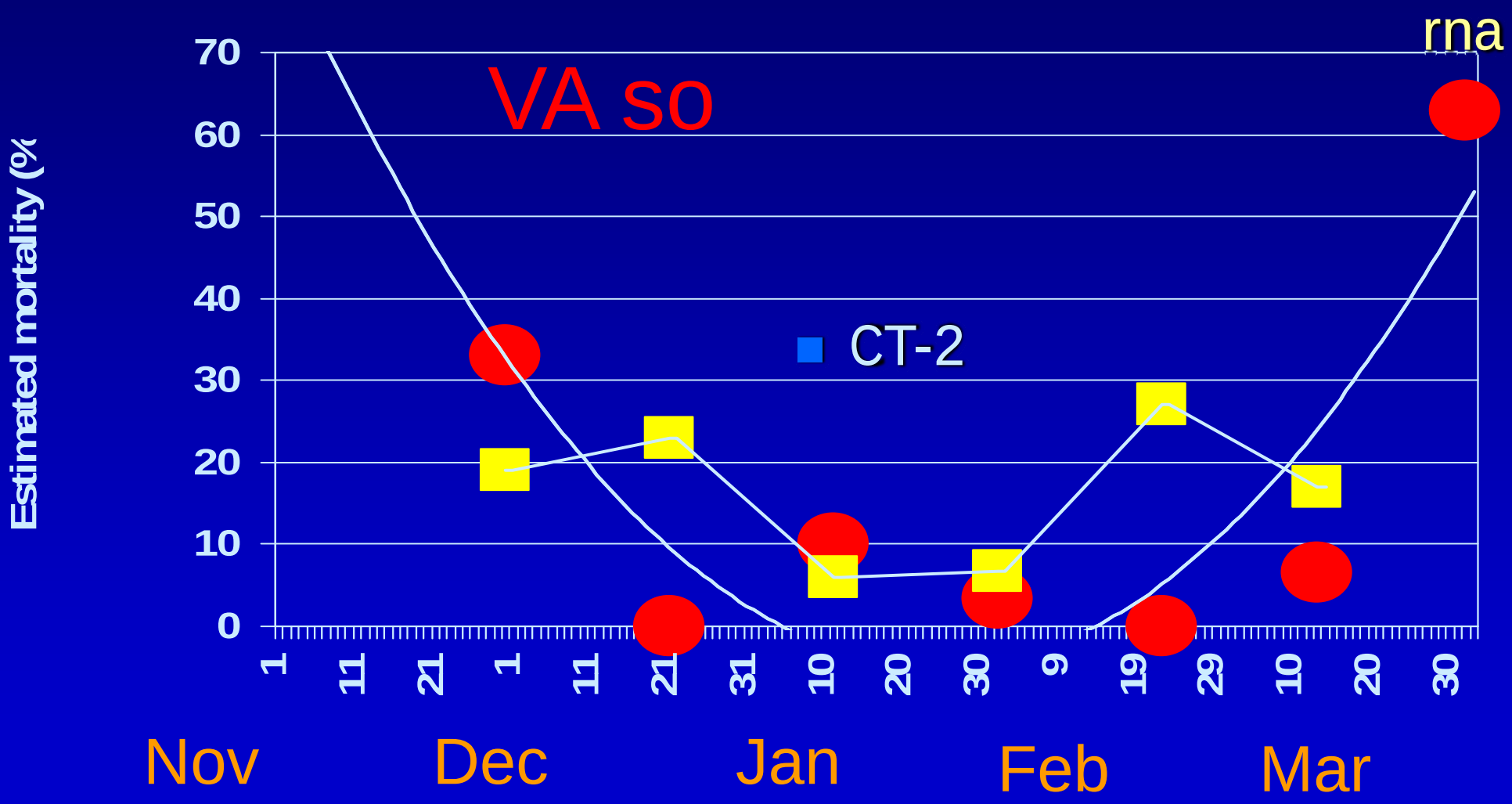
Correlations poor

- In general it seems to be hard to correlate expression data of cold stress indicators with fluctuations in ELT values, both for mortality and needle damage.

Estimated mortality to -10 C freeze



Estimated mortality to -10 C freeze



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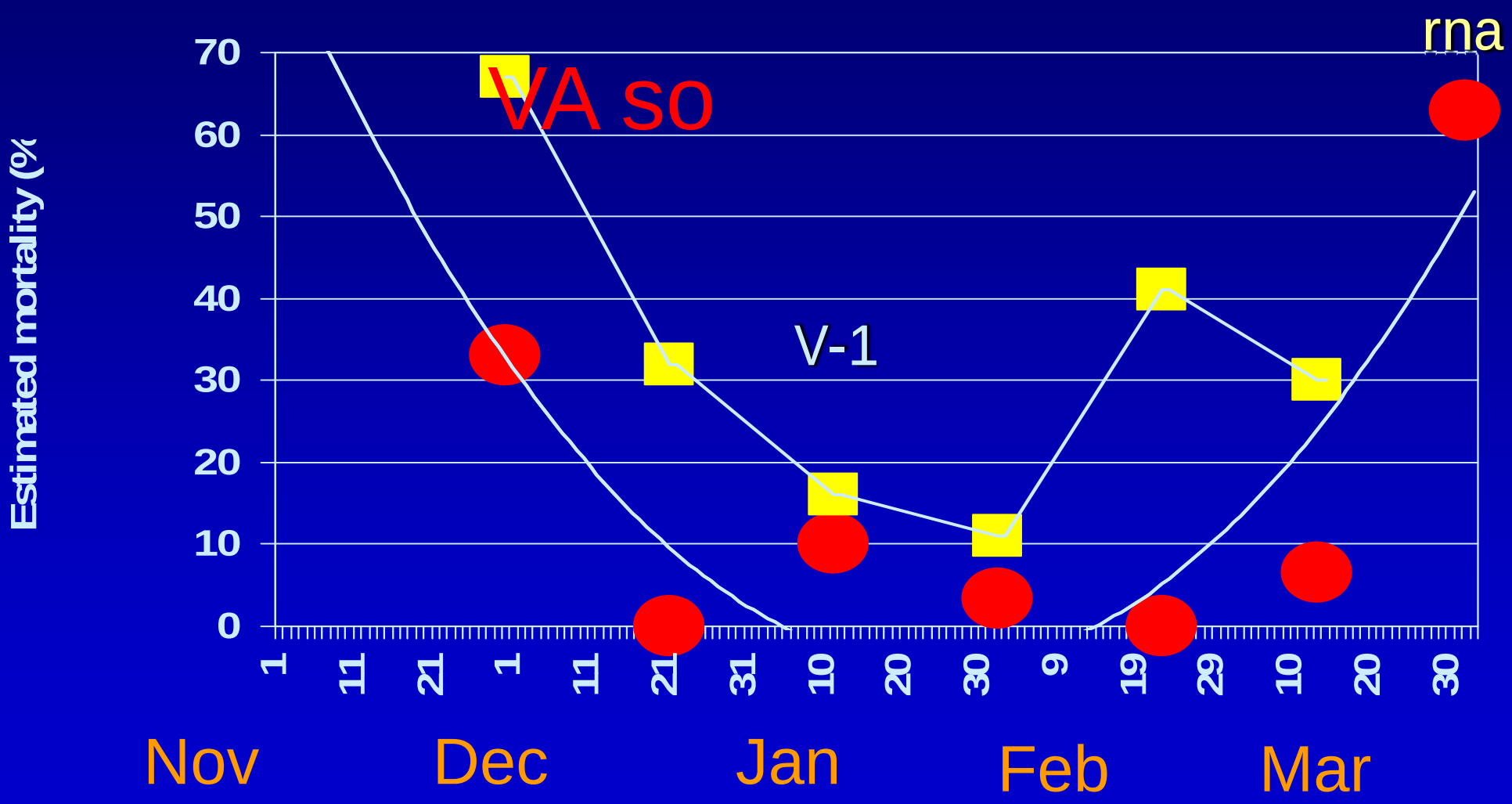
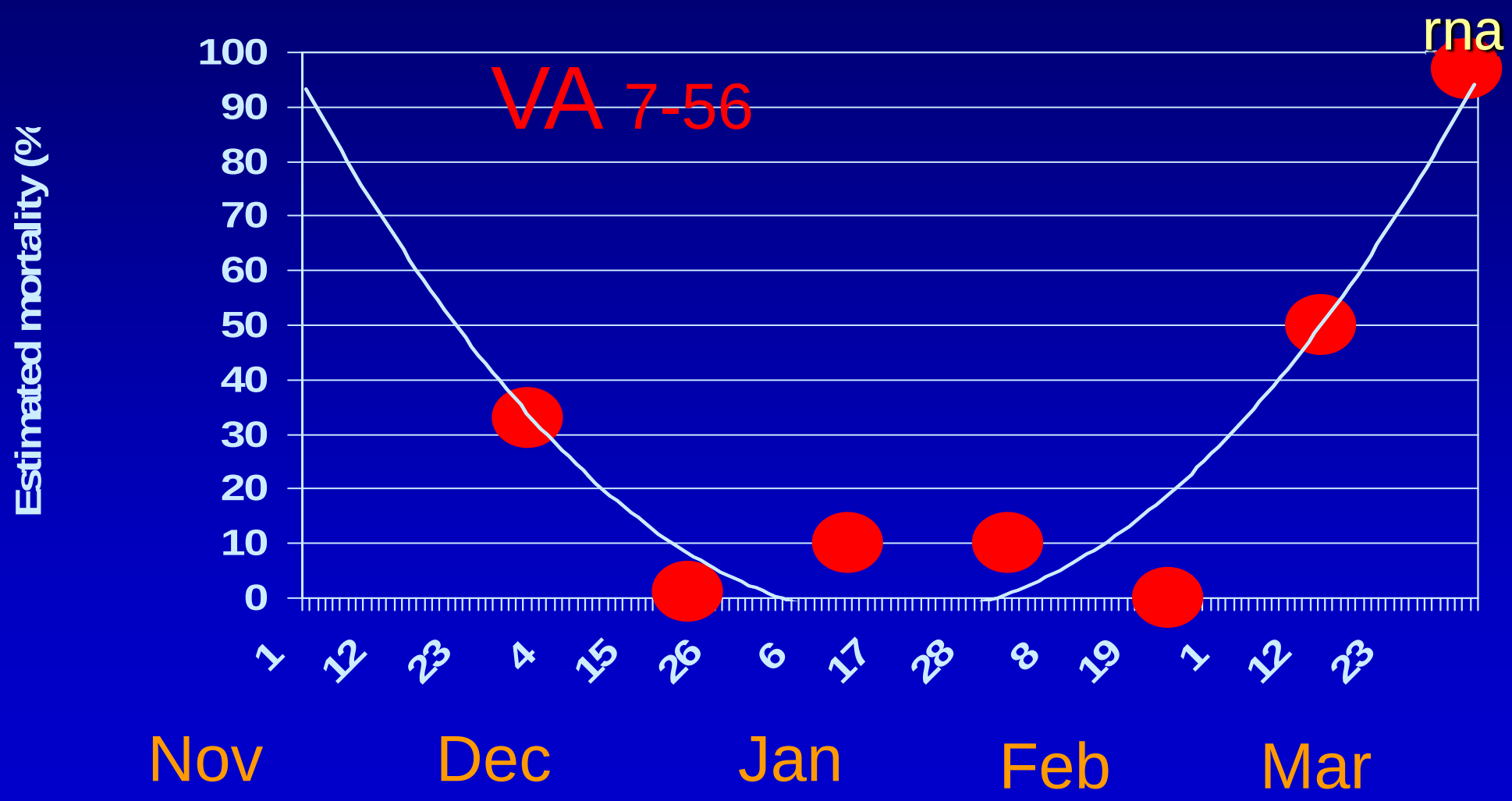


Estimated mortality to -10 C freeze



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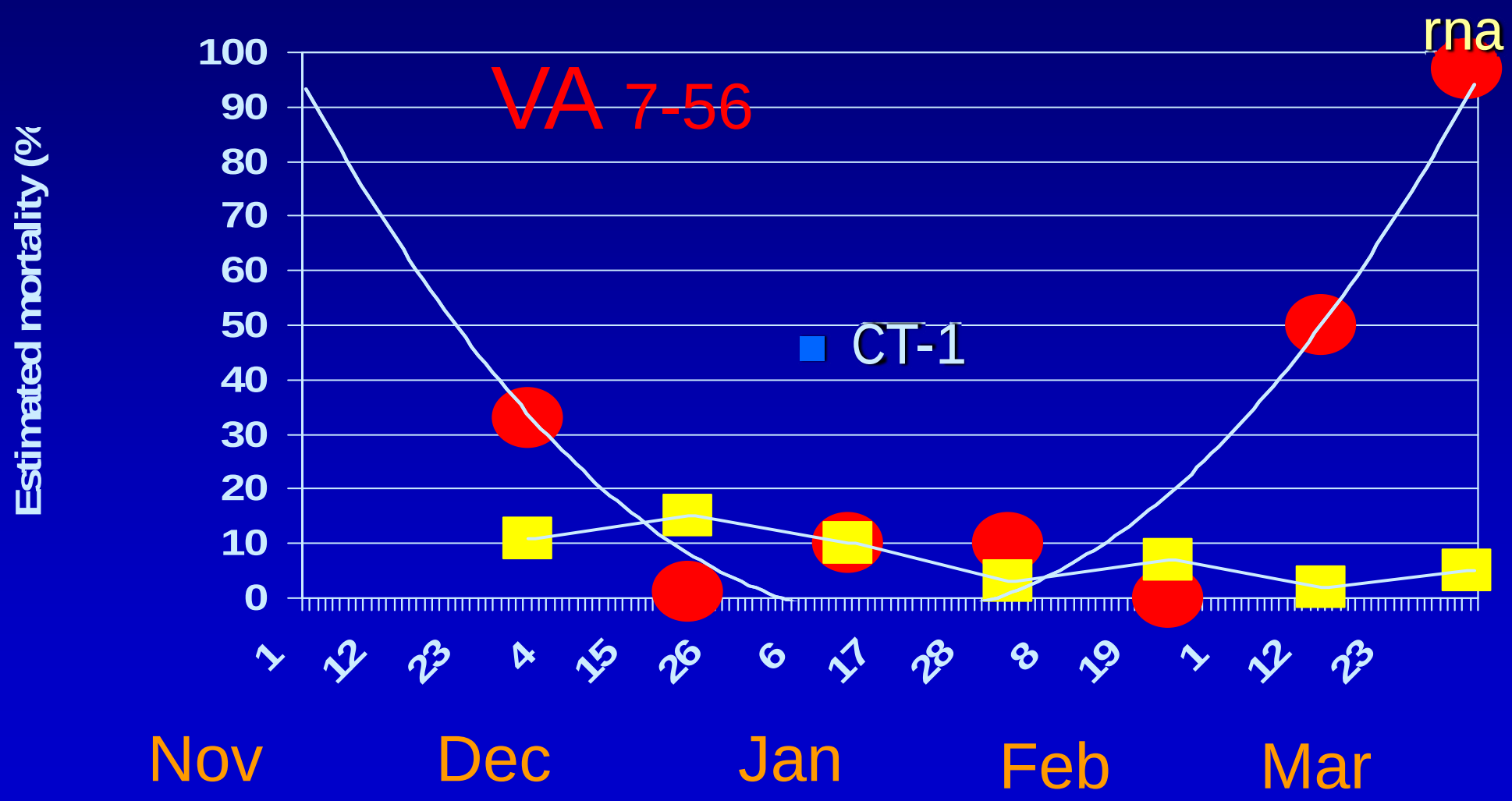
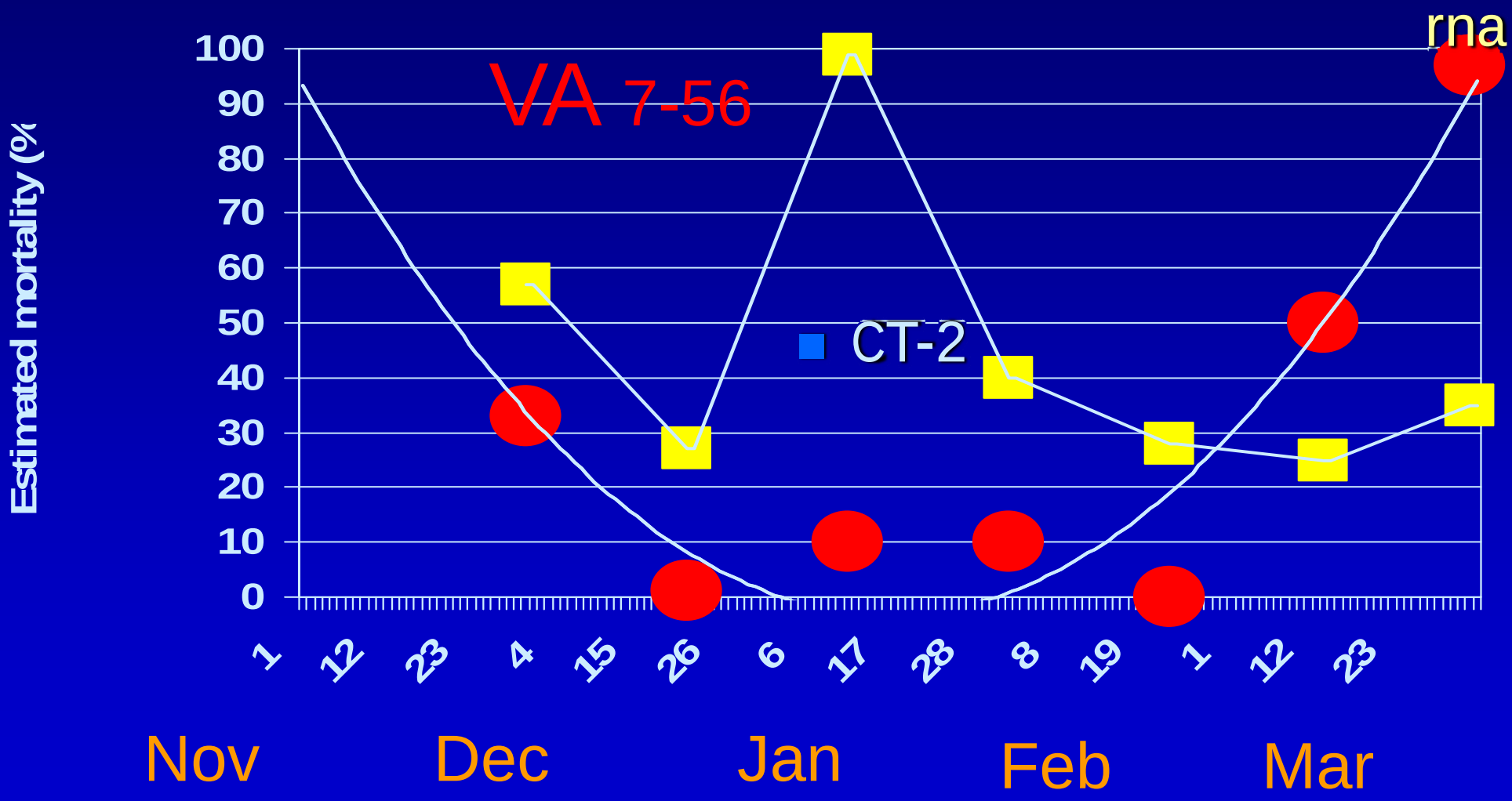
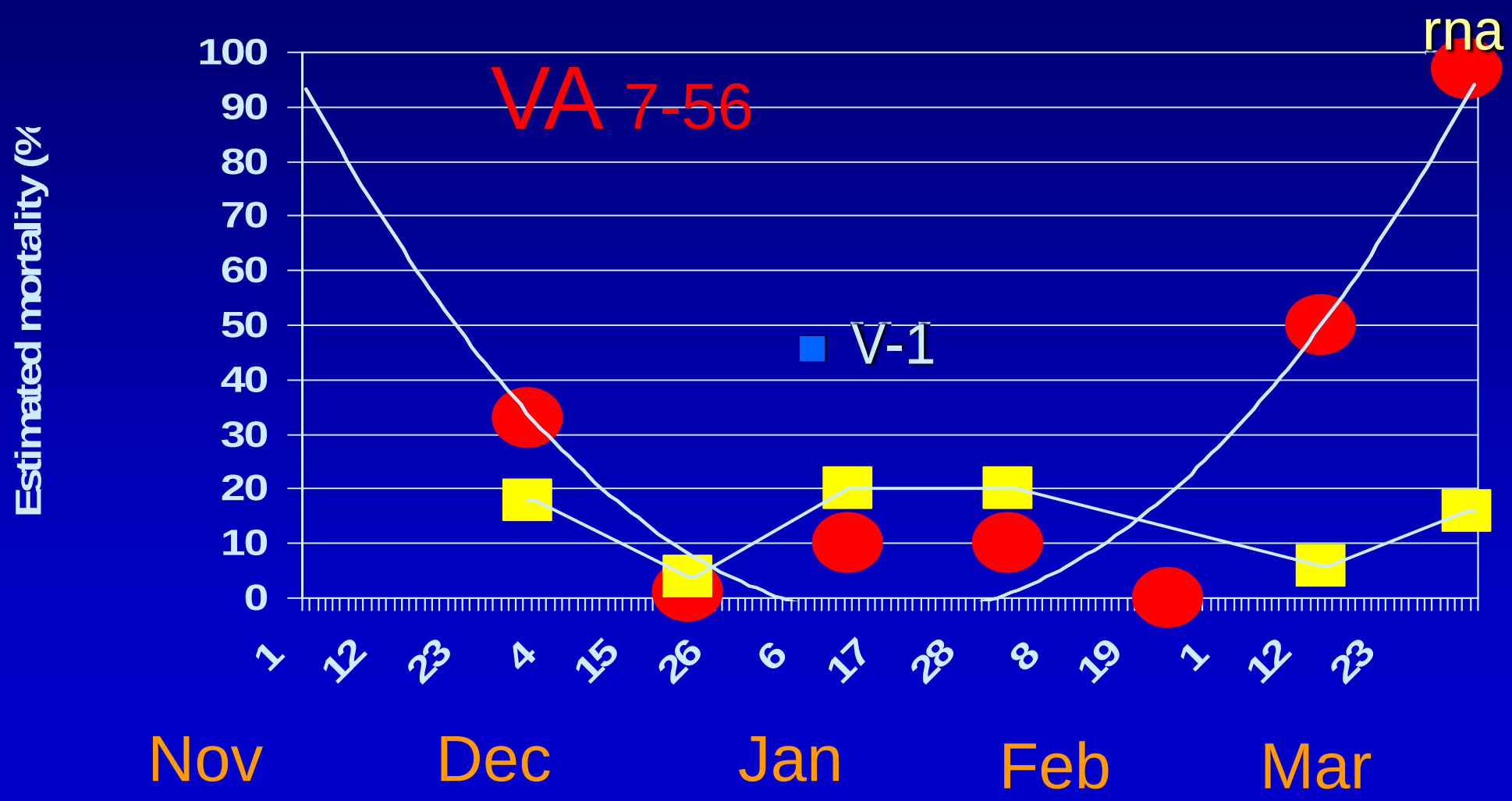


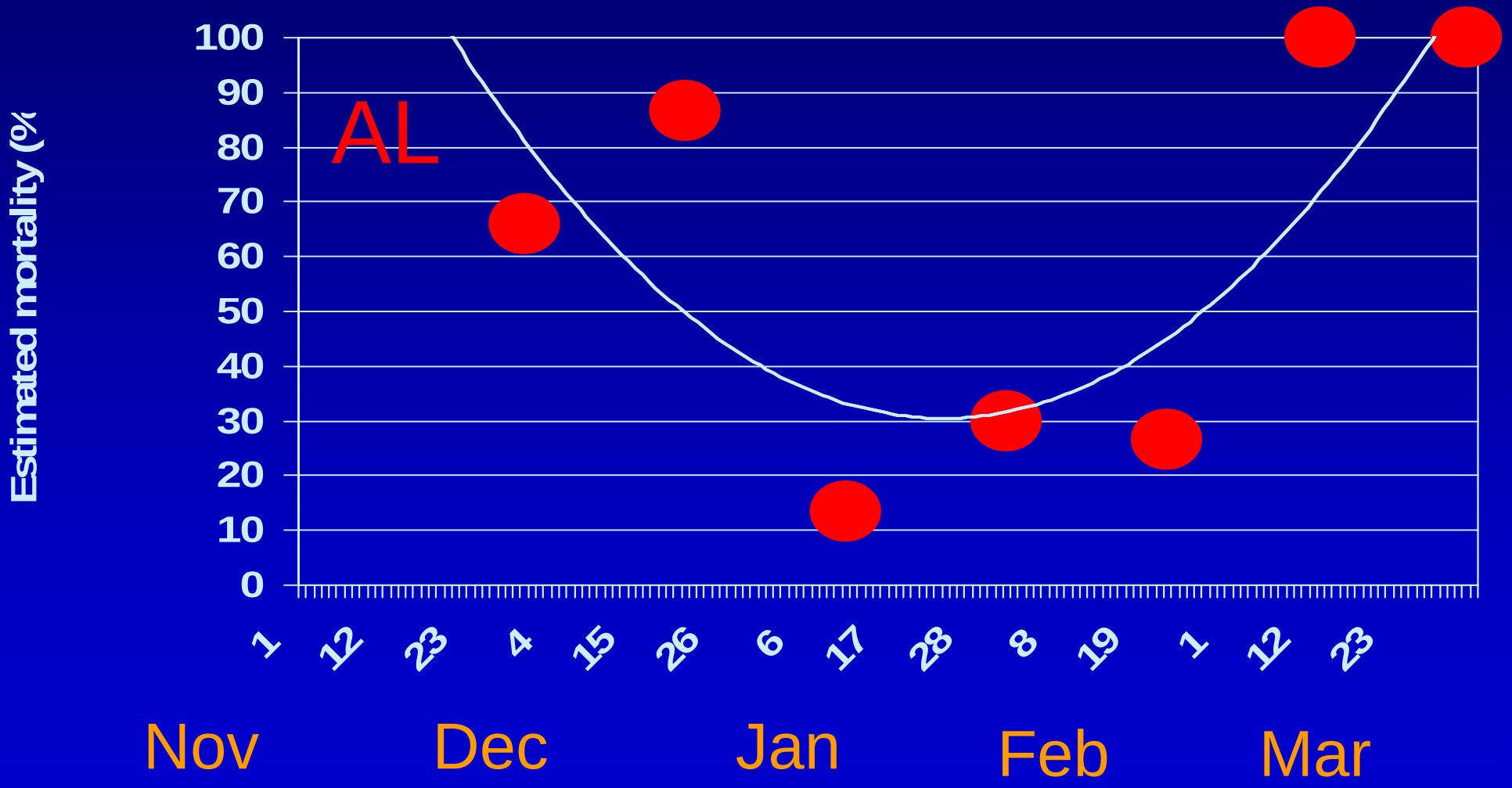
Estimated mortality to -10 C freeze



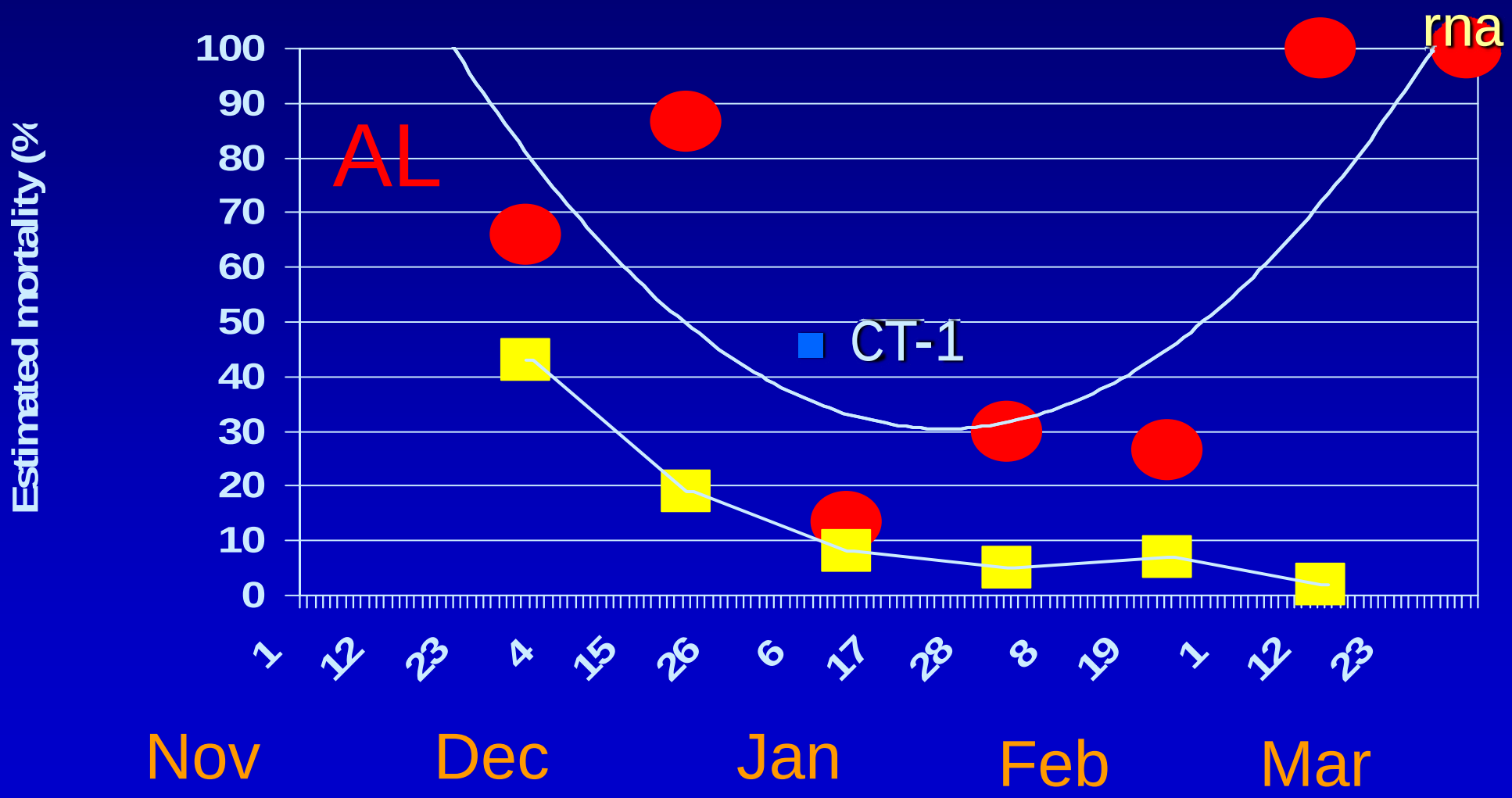
Estimated mortality to -10 C freeze



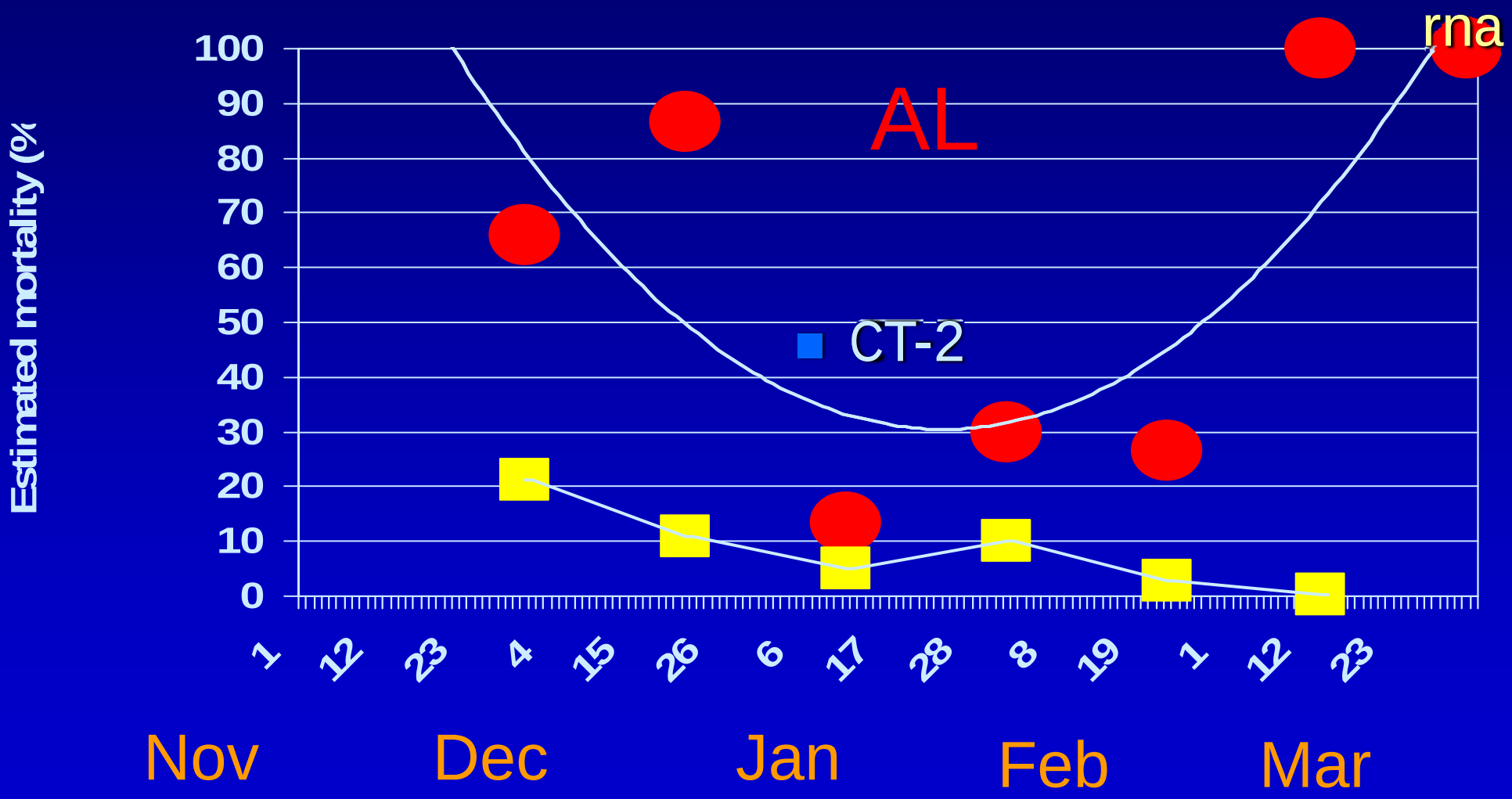
Estimated mortality to -10 C freeze



Estimated mortality to -10 C freeze



Estimated mortality to -10 C freeze



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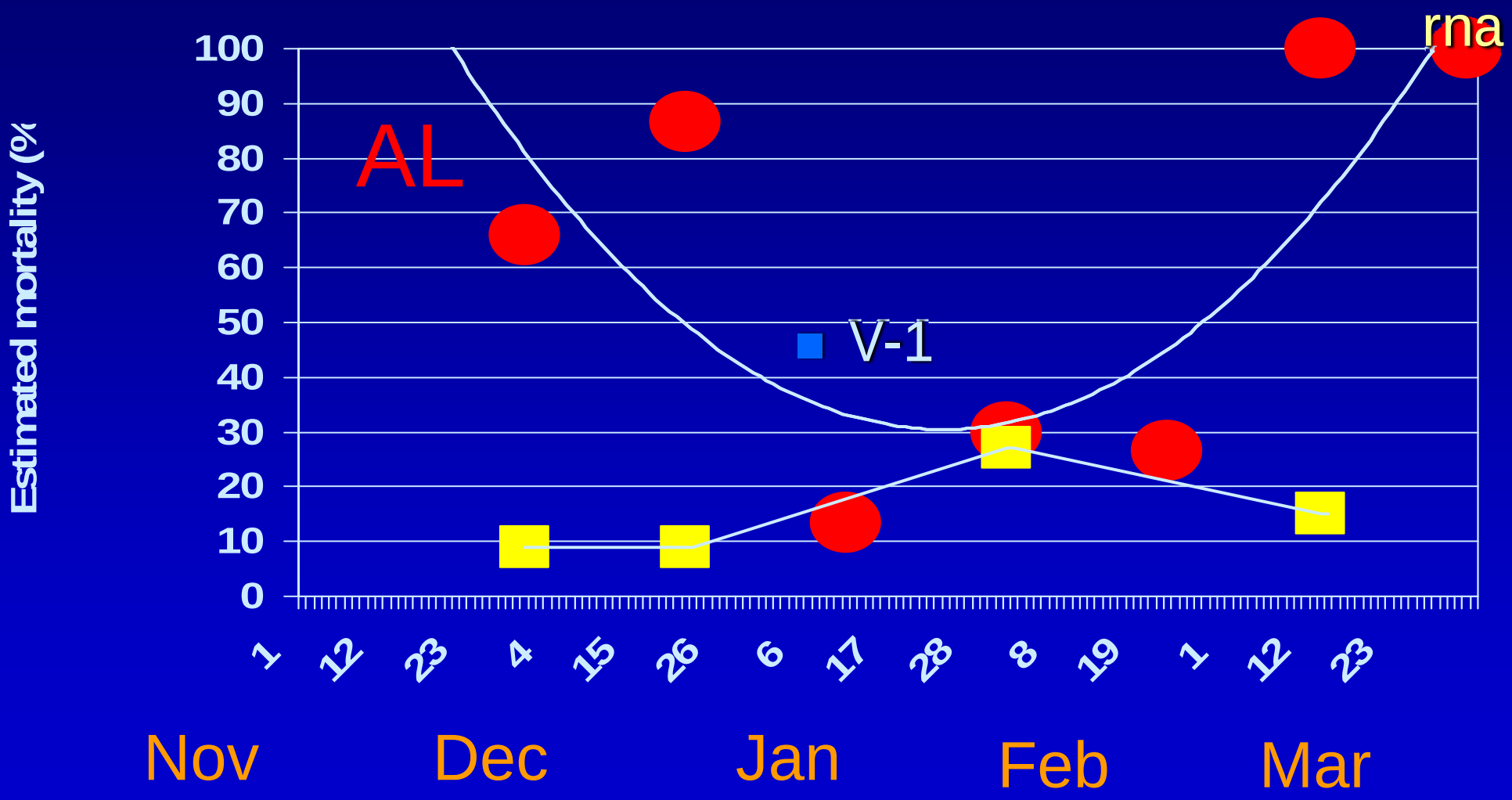


NSure Conclusions

- Focus should be on buds and stem.
- Samples should be analyzed shortly after collecting.
- A special extraction solution for highly contaminated samples should be used for tissues other than buds.
- More effort (money, some kind of funding) is needed to collect additional indicators, this study makes clear that is must be possible to find those.

Peter Balk
NSure bv, Wageningen
05 September 2008

Conclusions

- In agreement with findings with Douglas-fir, these results indicate that loblolly pine needles may not be a reliable test material.
- More effort is needed to evaluate additional indicators. This study indicates the current indicators (CT1, CT2 and V1) do not track cold hardiness of loblolly pine (as indicated by ELT).
- When rapid analysis is required, a cold-hardy test using ELT can be used to produce a cold-hardy chart.

Introductory price


As an introduction ColdNSure can this season be ordered at a 15% reduced price. The price for one test is not €125, but only €105!

Back to overview →

Orderinfo ColdNSure Pseudotsuga

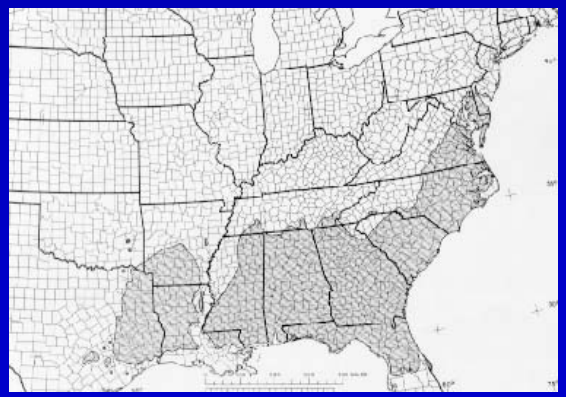
Option 1	**Sampling kit and analysis (1)** Order number: CNPM-S01	€ 105	**Order**
Option 2	**Sampling kit and analysis (5)** Order number: CNPM-S05	€ 485	**Order**
Option 3	**Sampling kit and analysis (10)** Order number: CNPM-S10	€ 935	**Order**

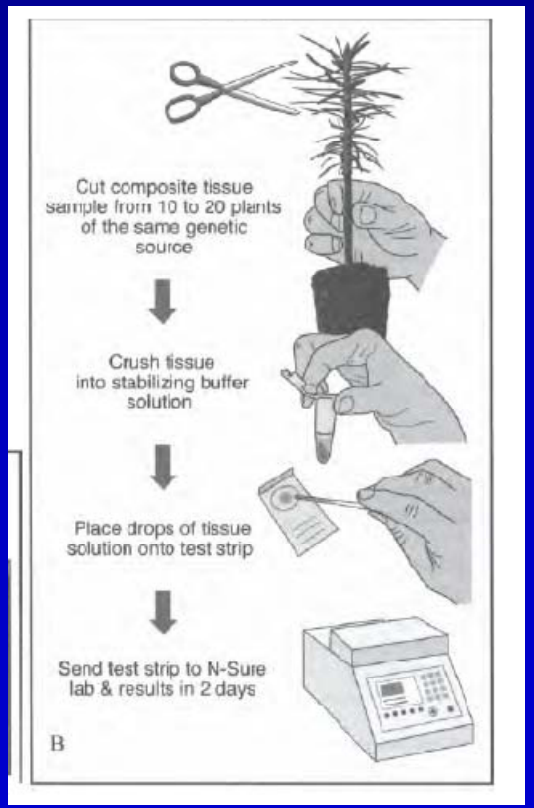
- $133
- $123
- $119

Do we need results in 2 days?

Test type		Duration of test (lapse time)
Morphology	Height, RCD, usability	<1 days
	Root:shoot ratio	4
Root electrolyte leakage	**Cold NSure**	2
Shoot electrolyte leakage		2
Root moisture content		4
Root growth potential		28

Cold NSure staff would like to know if anyone would be interested in cooperating in a follow-up trial.





Thanks to...

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Questions?