



Cryopreservation of dormant buds

General principles and practical approaches

Crop Research Institute, Prague, Czech Republic

Alois Bilavcik

Training School on Dormant Bud Cryopreservation, 21-23 May 2024, Faenza, Italy



3rd Workshop on Cryopreservation by the Dormant-Bud Technique,
13-14 December 2012, Crop Research Institute, Prague, Czech Republic



GENERAL PRINCIPLES

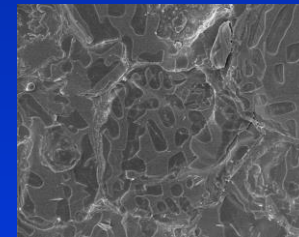
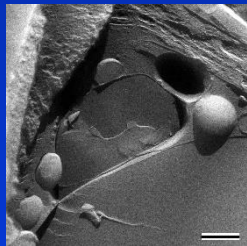


Survival of plant cells below their melting point

„Amorphousness“

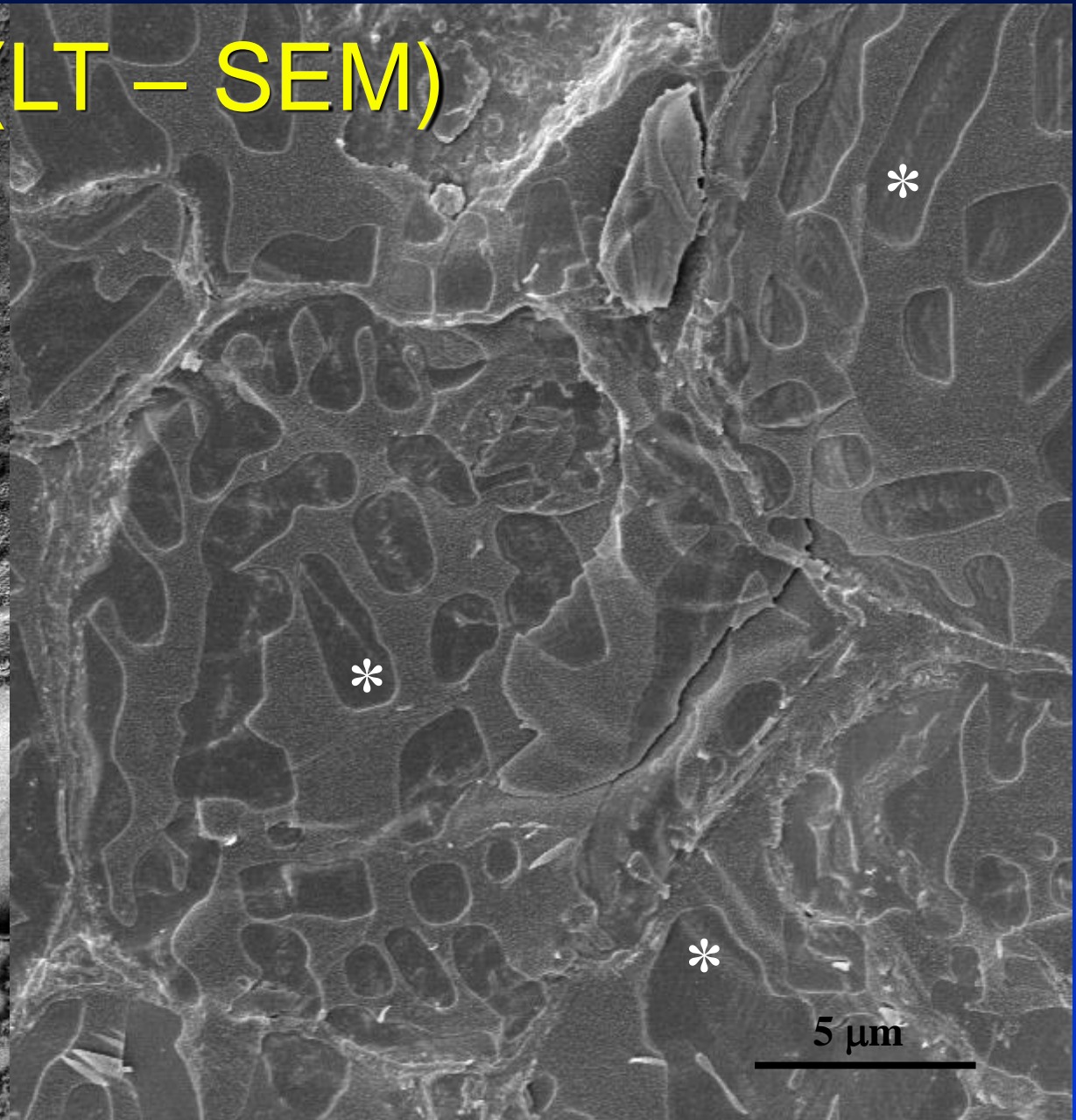
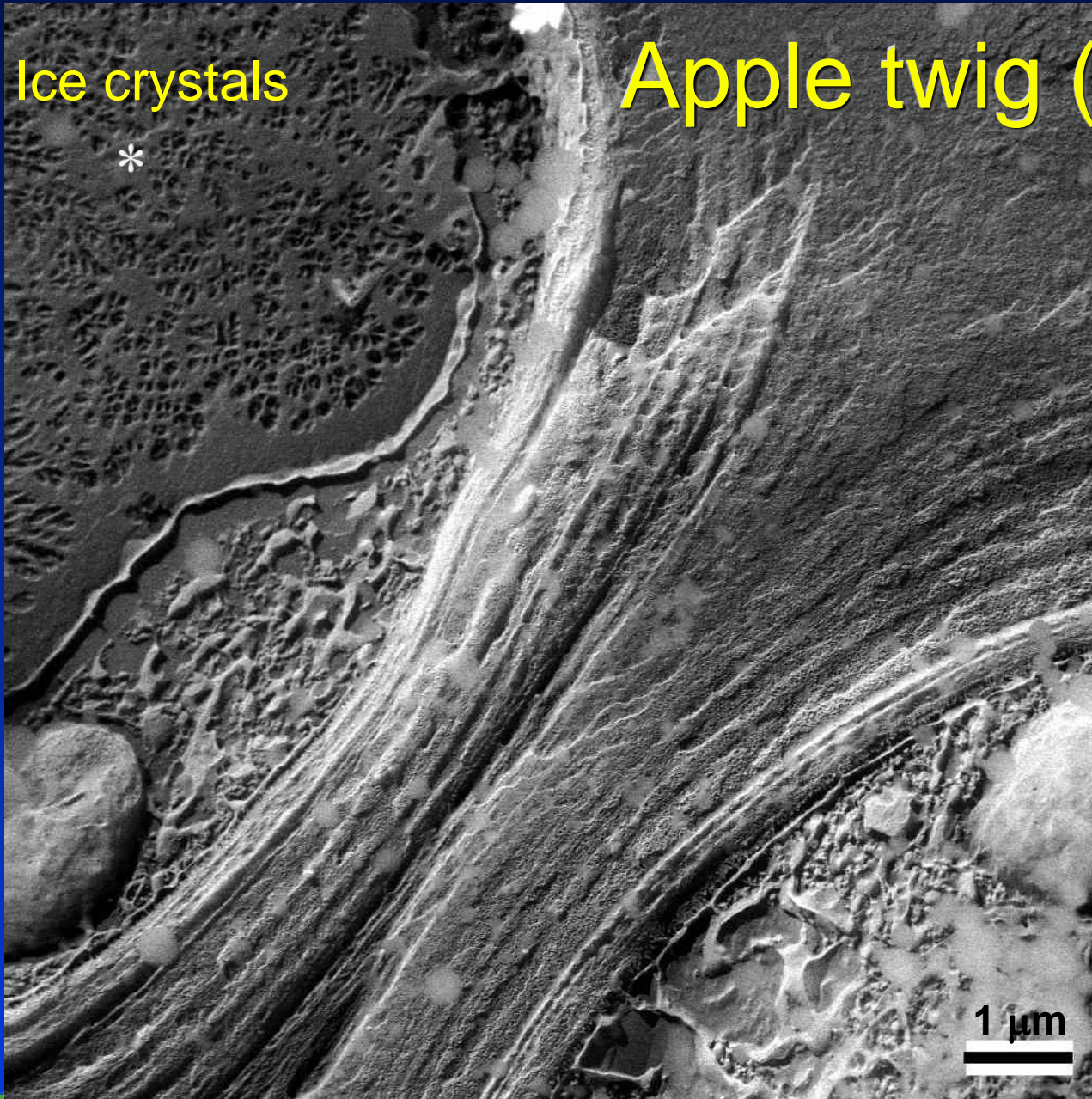
X

Ice crystals

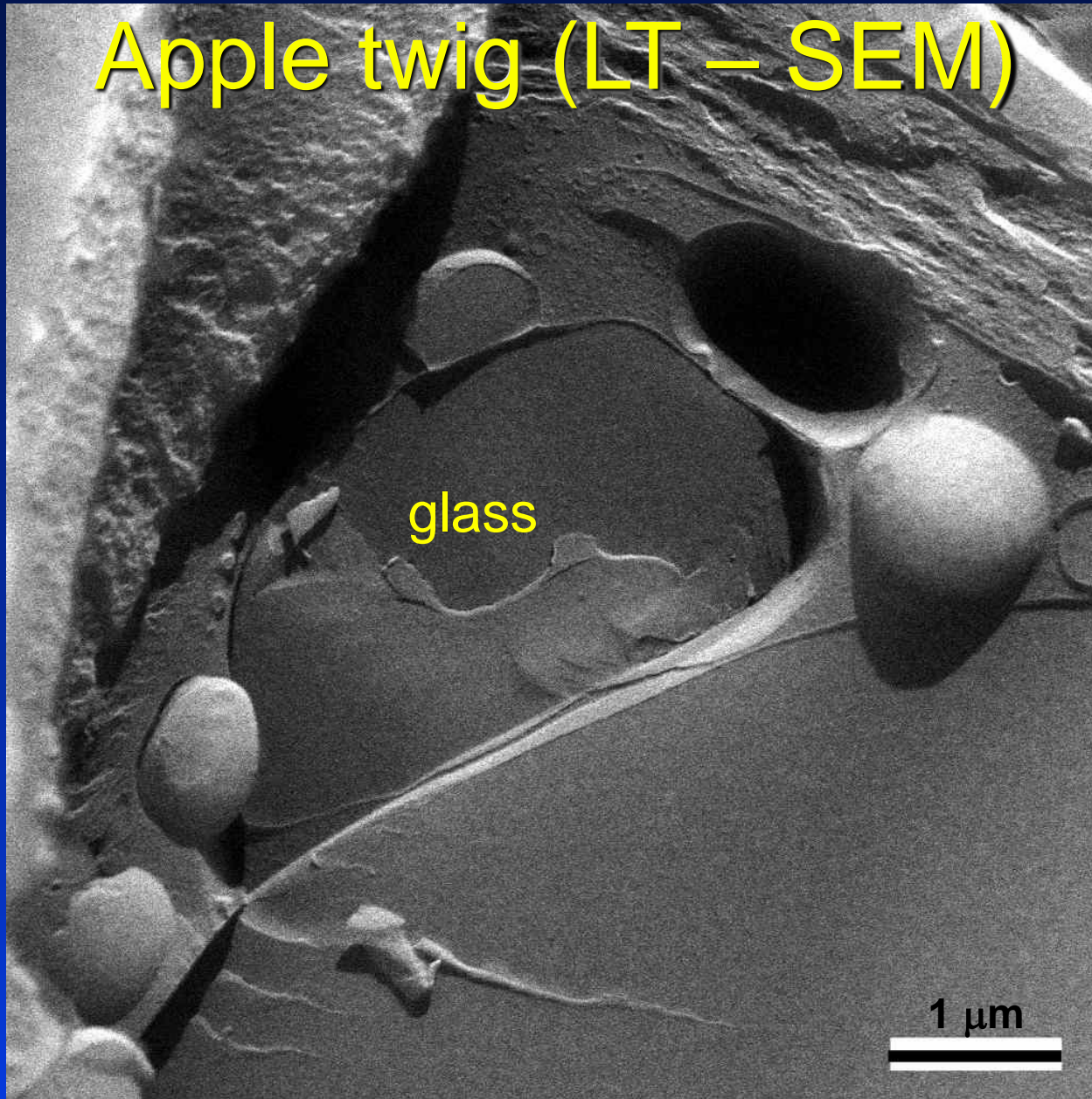


Ice crystals

Apple twig (LT – SEM)

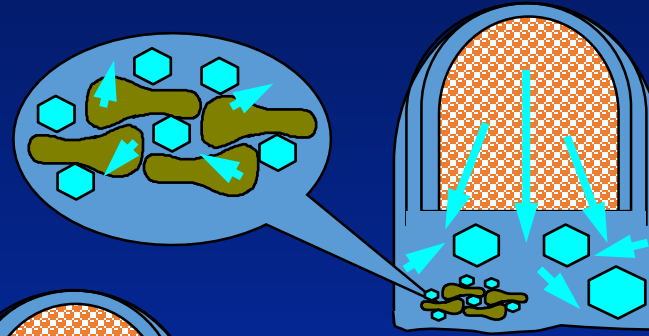


Apple twig (LT – SEM)

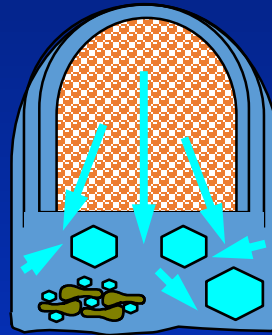


Physiology of bud survival *ex situ* „thermal strategy“

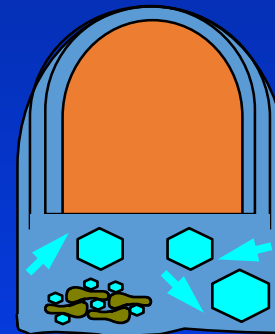
- Extracellular freezing



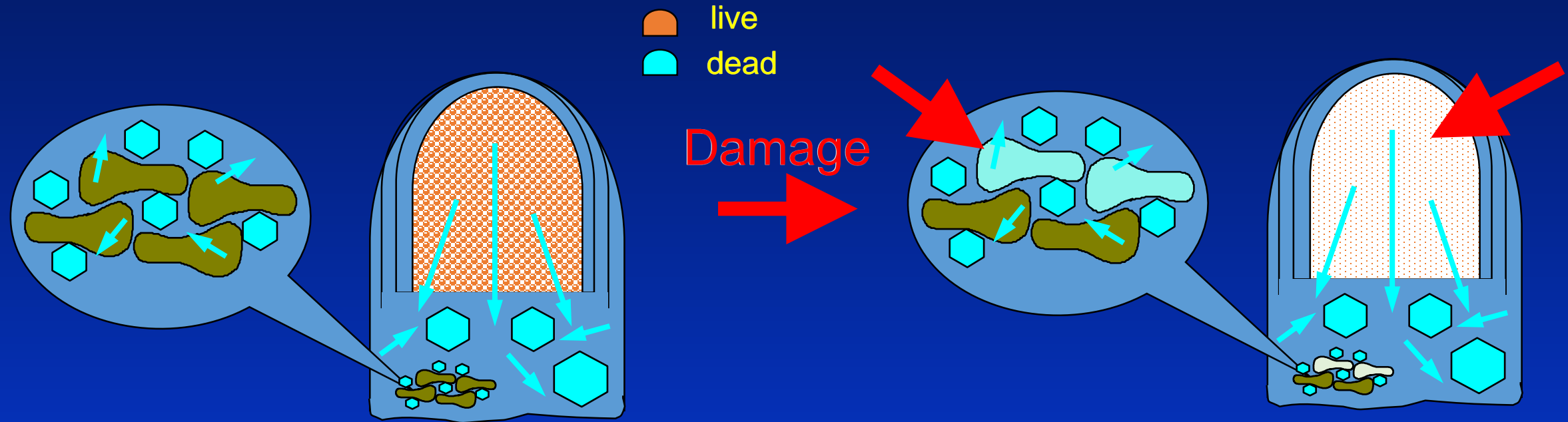
- Extraorgan freezing



- Supercooling (limit to ~ -40 °C)

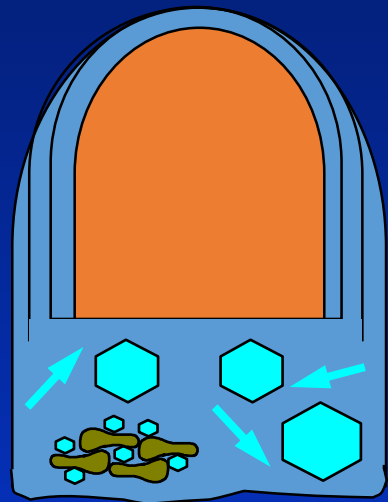


Extracellular & Extraorgan freezing

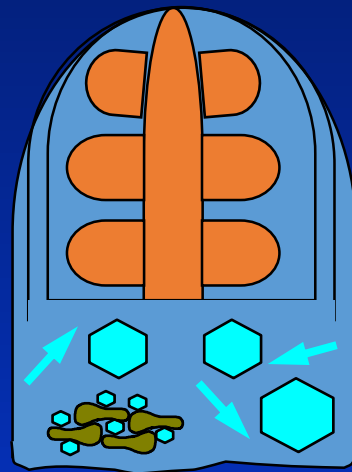


Extracellular freezing & supercooling

live
dead

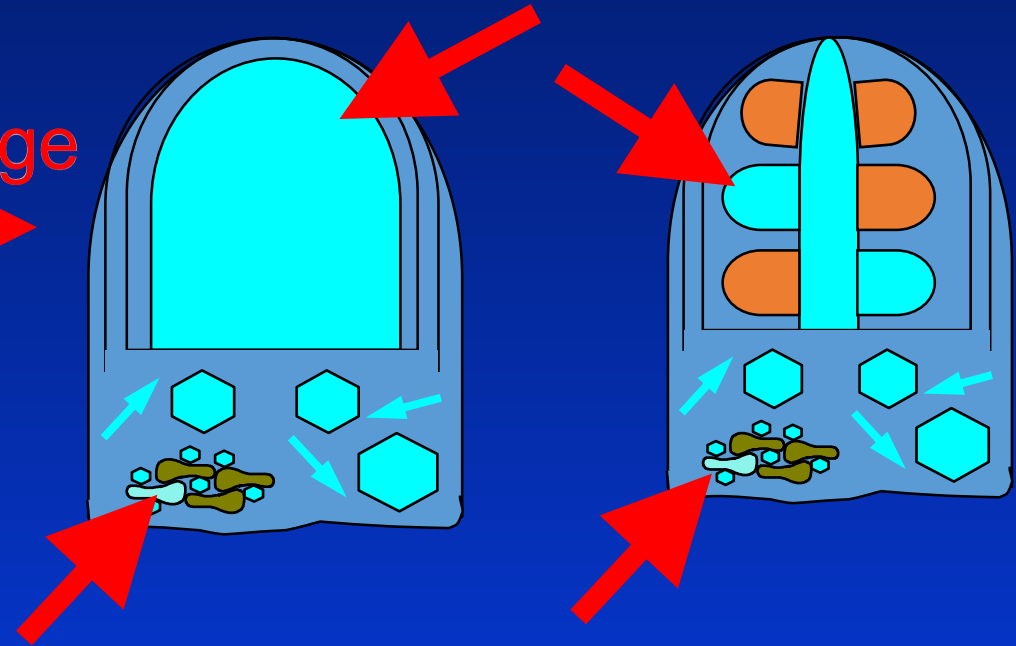


Peach



Grapevine

Damage



Cryopreservation → induction of glass formation

- Physical pretreatment
 - dehydration
 - freezing/warming rate
- Cryoprotectant pretreatment
 - sugars, polyols, hydrophilic proteins,...



OPTIMAL ORCHARD SAMPLING



Sampling conditions

- Optimal conditions
 - Date – December - February
(ecodormant)
 - Temperature – longer period of subzero temperatures
 - Keeping – temperature $-4\text{ }^{\circ}\text{C}$
- Suboptimal conditions
 - Hardening of plant material



Blue honeysuckle sampling



Mendel University Agriculture Enterprise in Žabčice, Czech Republic



Blackcurrant sampling

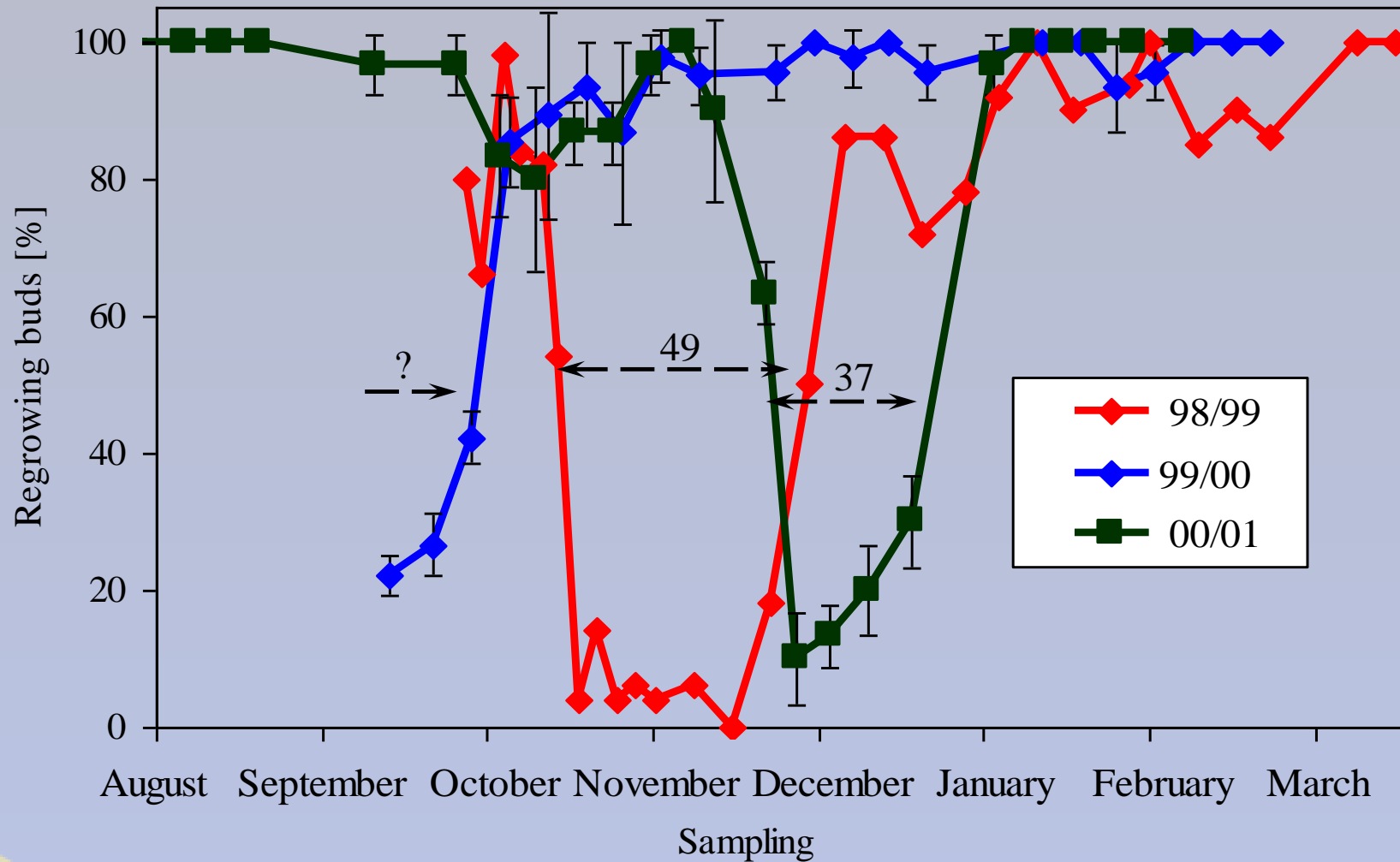


Pavel Res Farm, Bratkovice, Czech Republic.
With the mythical Czech mountain Říp on the horizon.

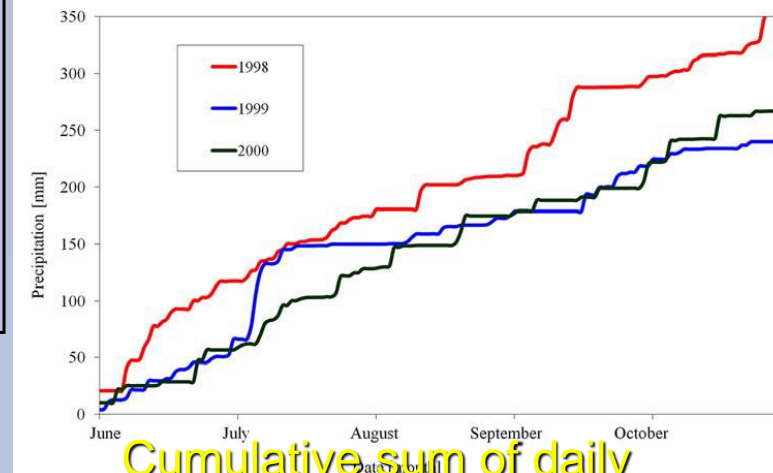
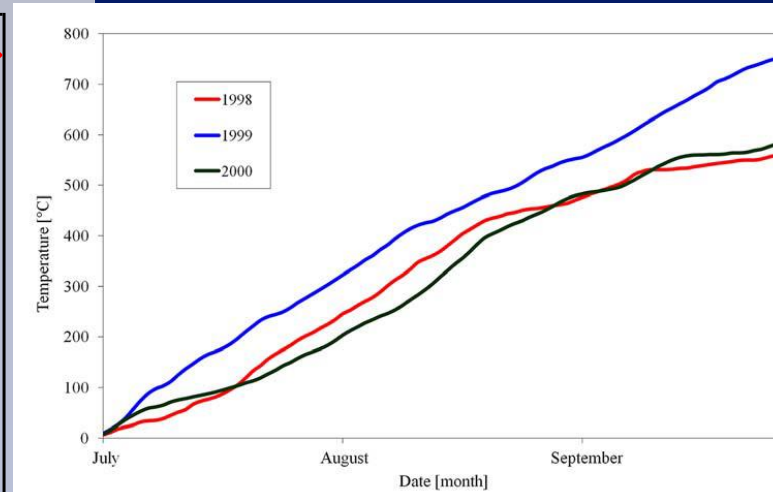


Course of apple shoots endodormancy

Endodormancy - 'Šampion'



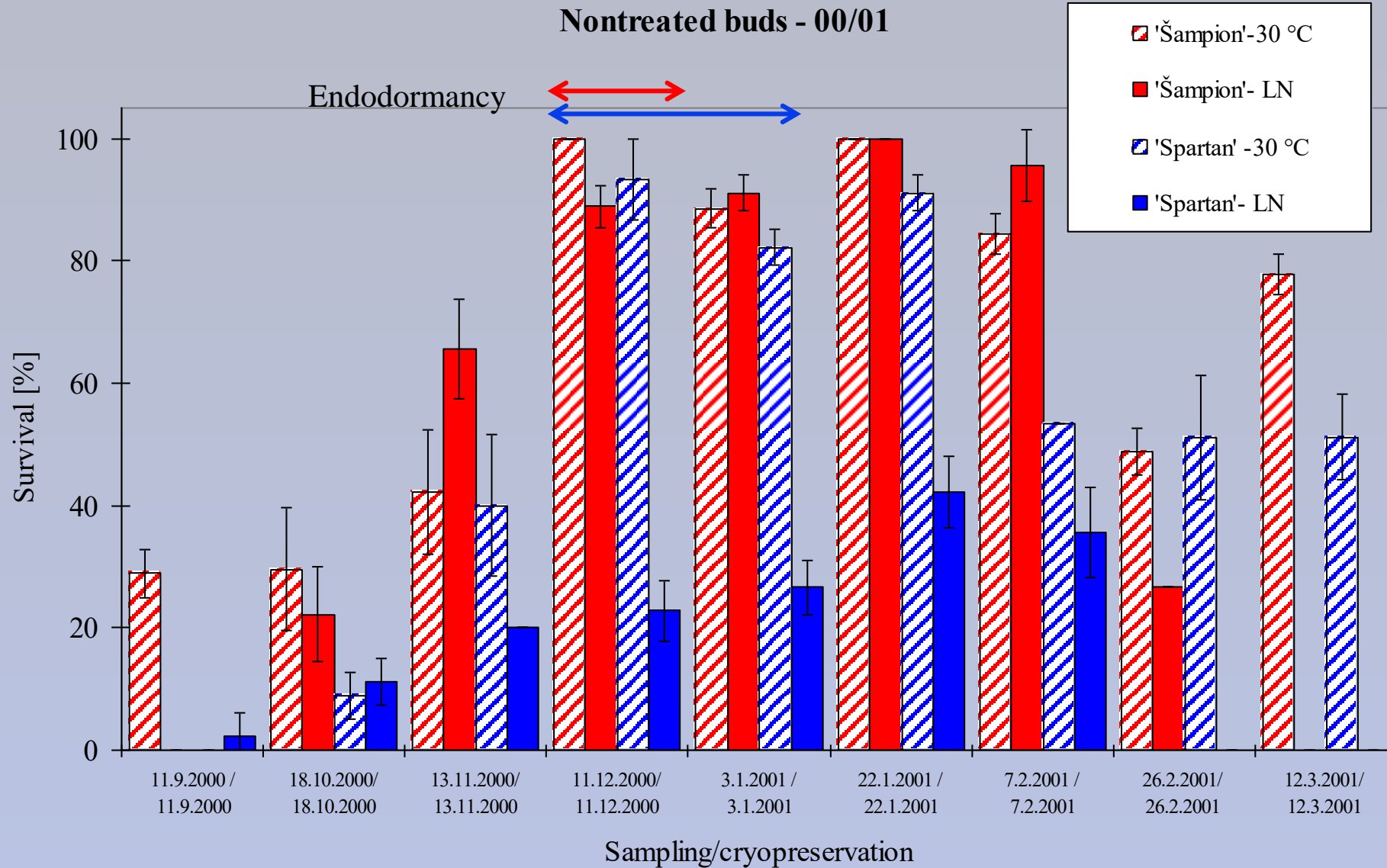
Cumulative sum of average daily temperatures above 10 °C



Cumulative sum of daily precipitations (from June)



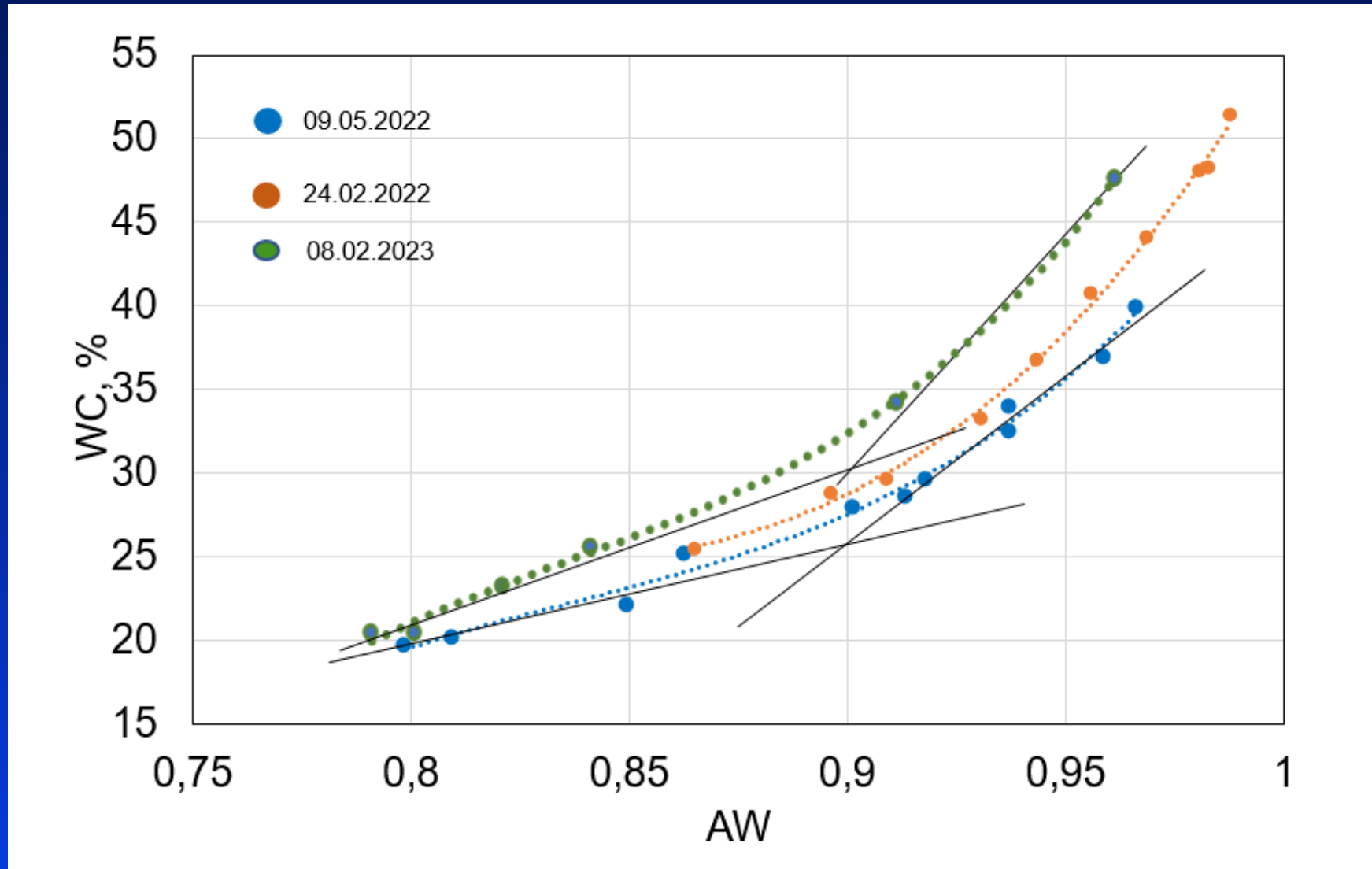
Course of apple tree low temperature survival



FROST DEHYDRATION & THERMAL CHARACTERISATION



Course of frost dehydration dormant raspberry buds - different seasons



Thermal characterisation of dormant raspberry buds

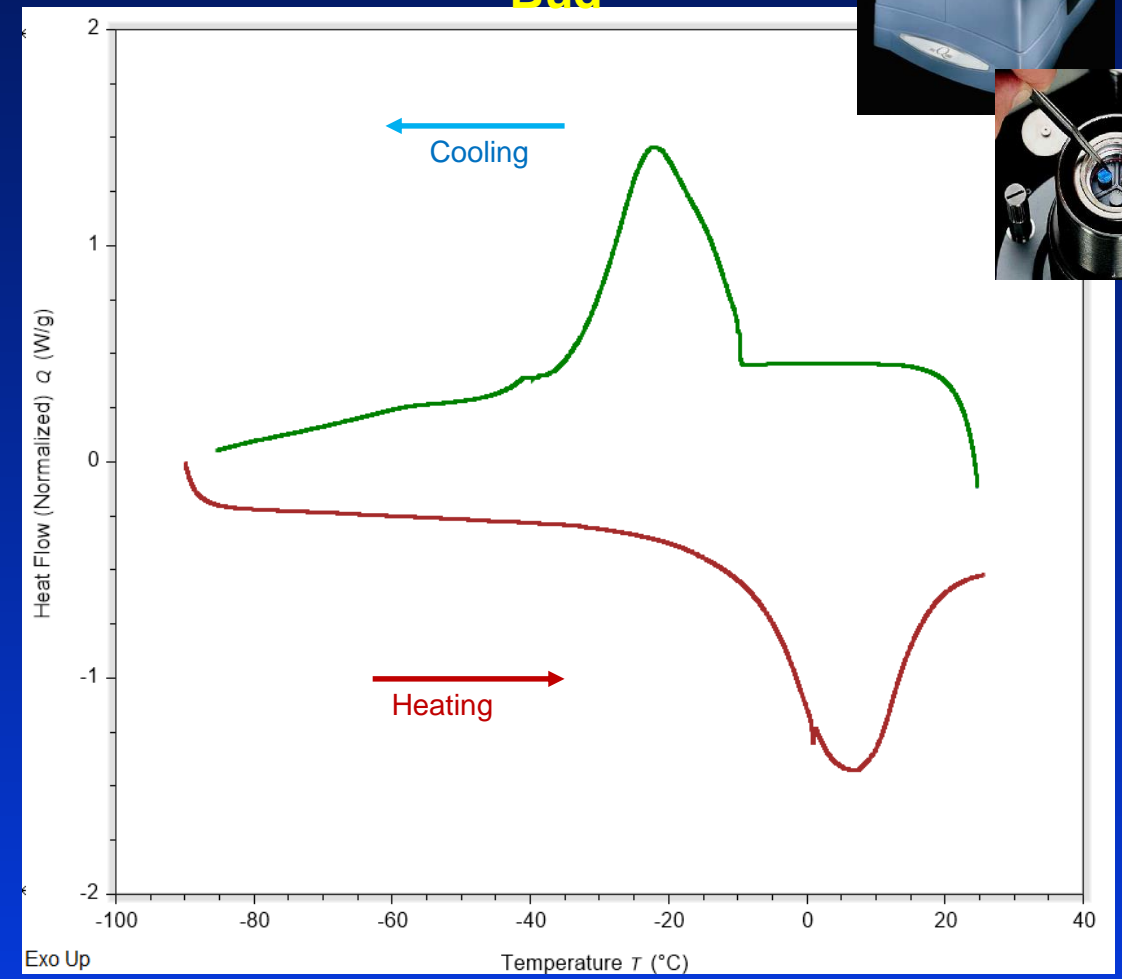
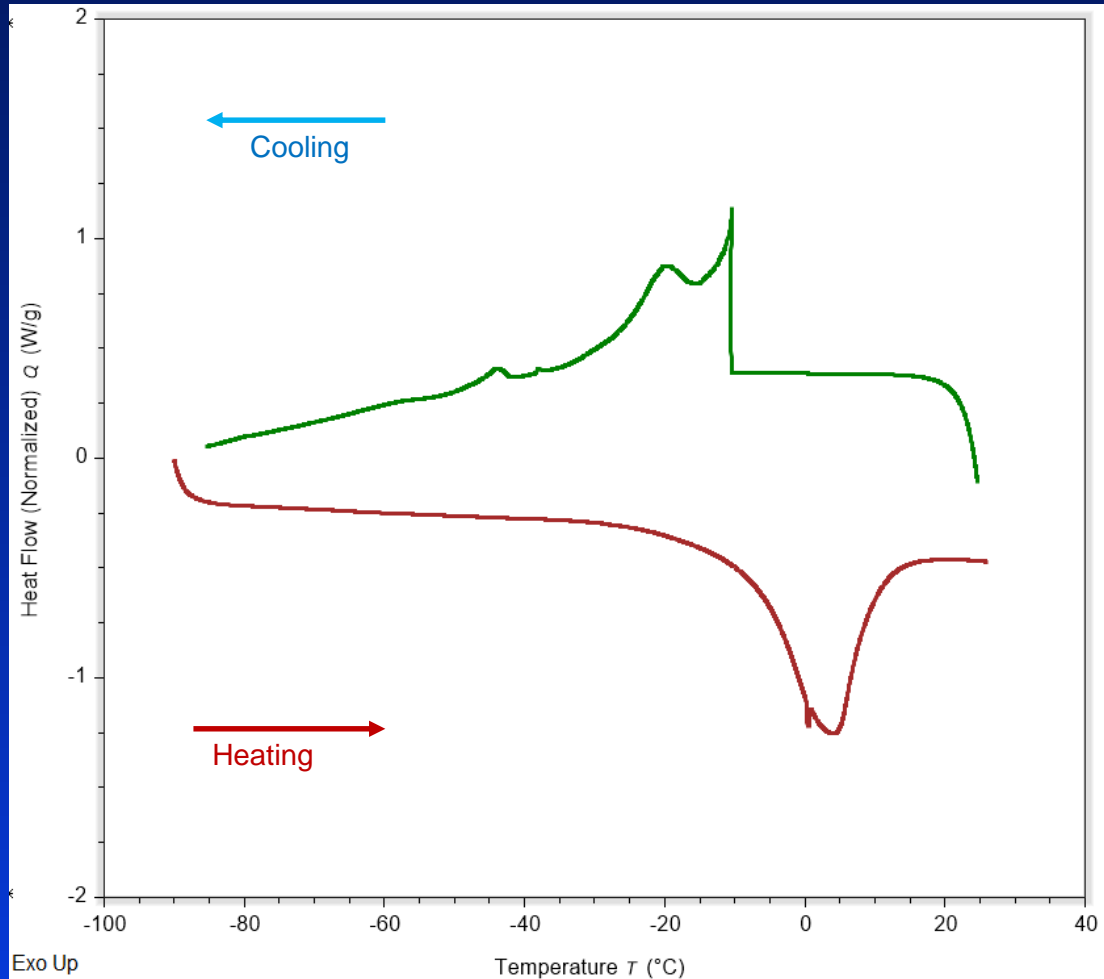
Differential Scanning Calorimetry (DSC)



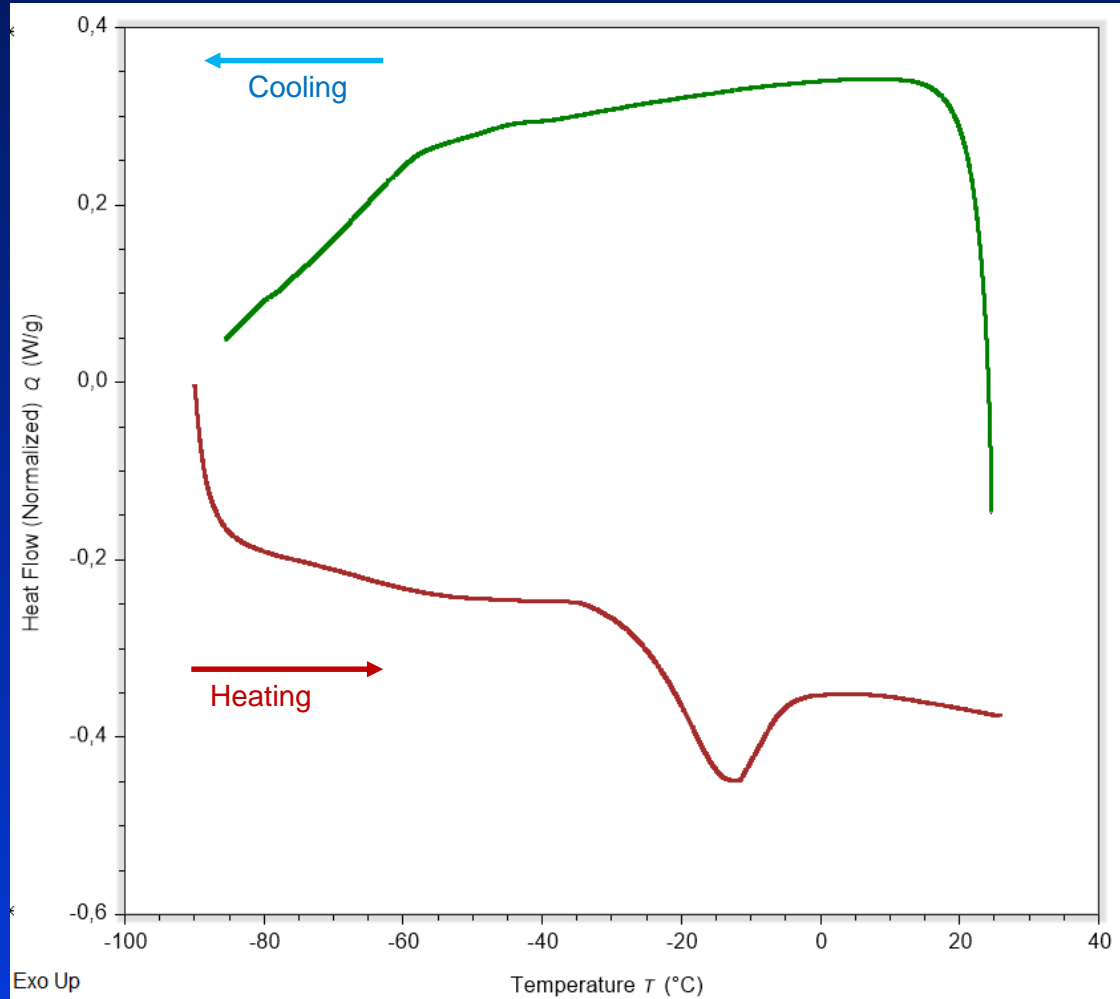
Internode

Fresh sample

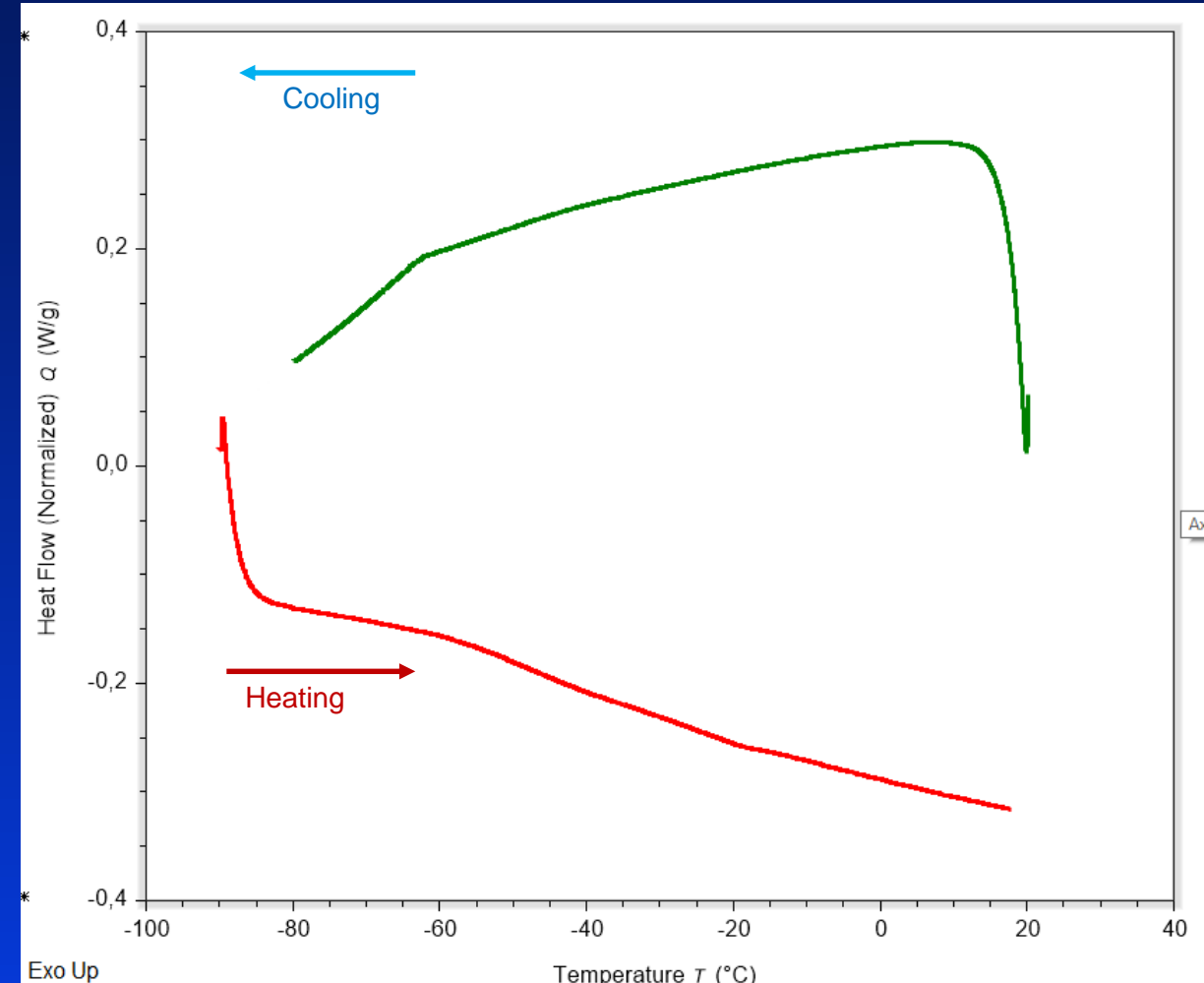
Bud



Phase transitions during dehydration dormant raspberry buds

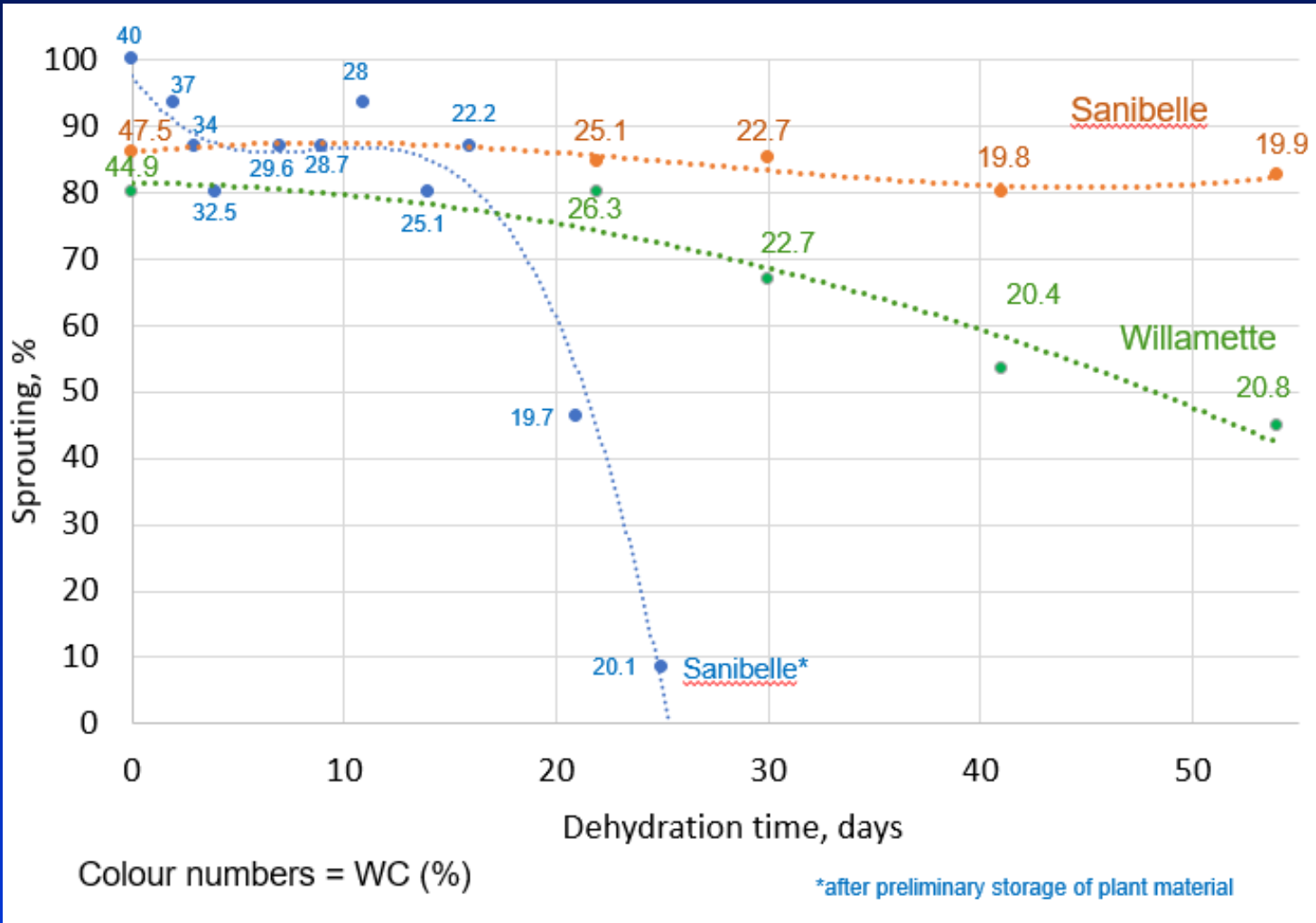


WC=26.3%
AW=0.87



WC=20.4%
AW=0.83

Regeneration of dormant raspberry buds after dehydration



Willamette
WC=20.8%

Sanibelle
WC=19.9%



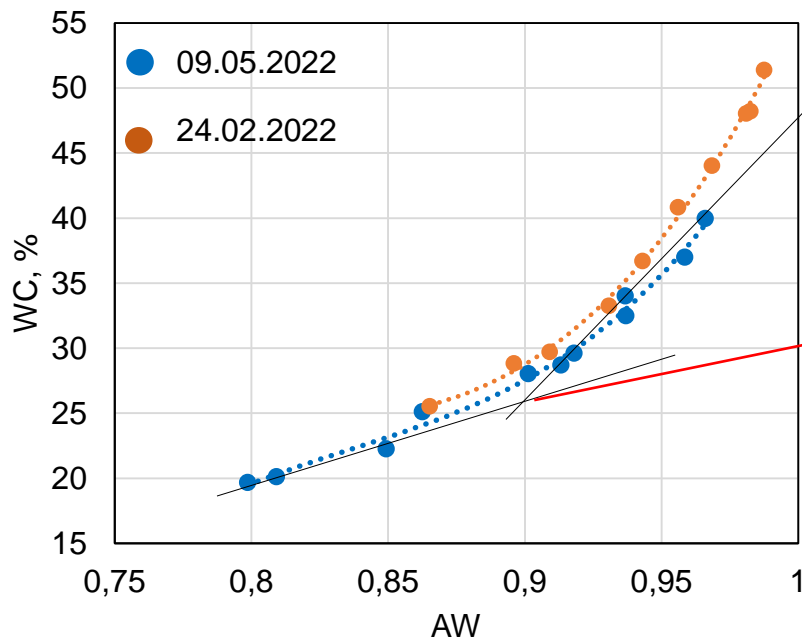
54 days of dehydration



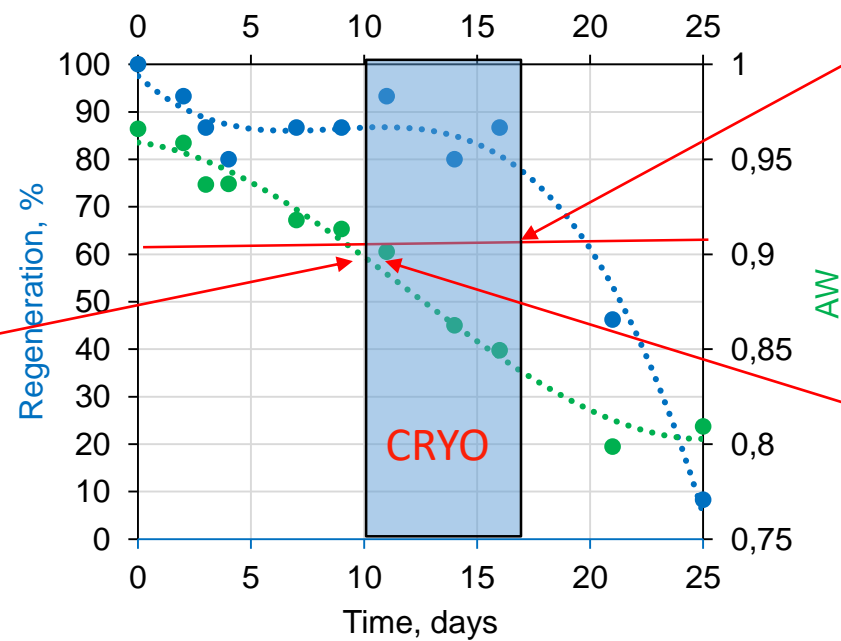
Dormant raspberry buds – dehydration settings

- Determination the 'breakpoint' in the dehydration curve (WC/aw - approx. 0.9)
- Correlation the crystallized water amount with the dehydration curve
- Determination the suitable dehydration range - "dehydration window" – according to the regeneration

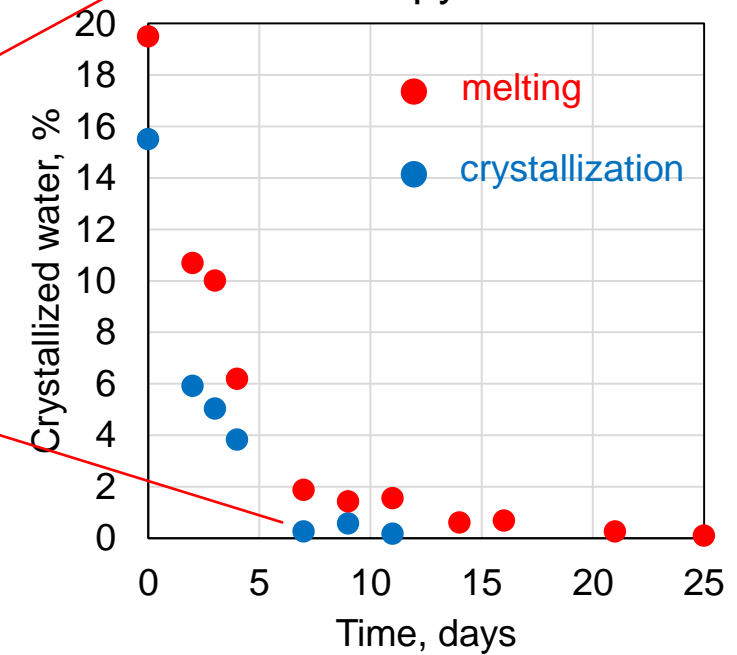
Water status



Dehydration - Regeneration and AW

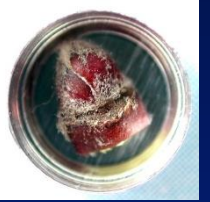


Enthalpy

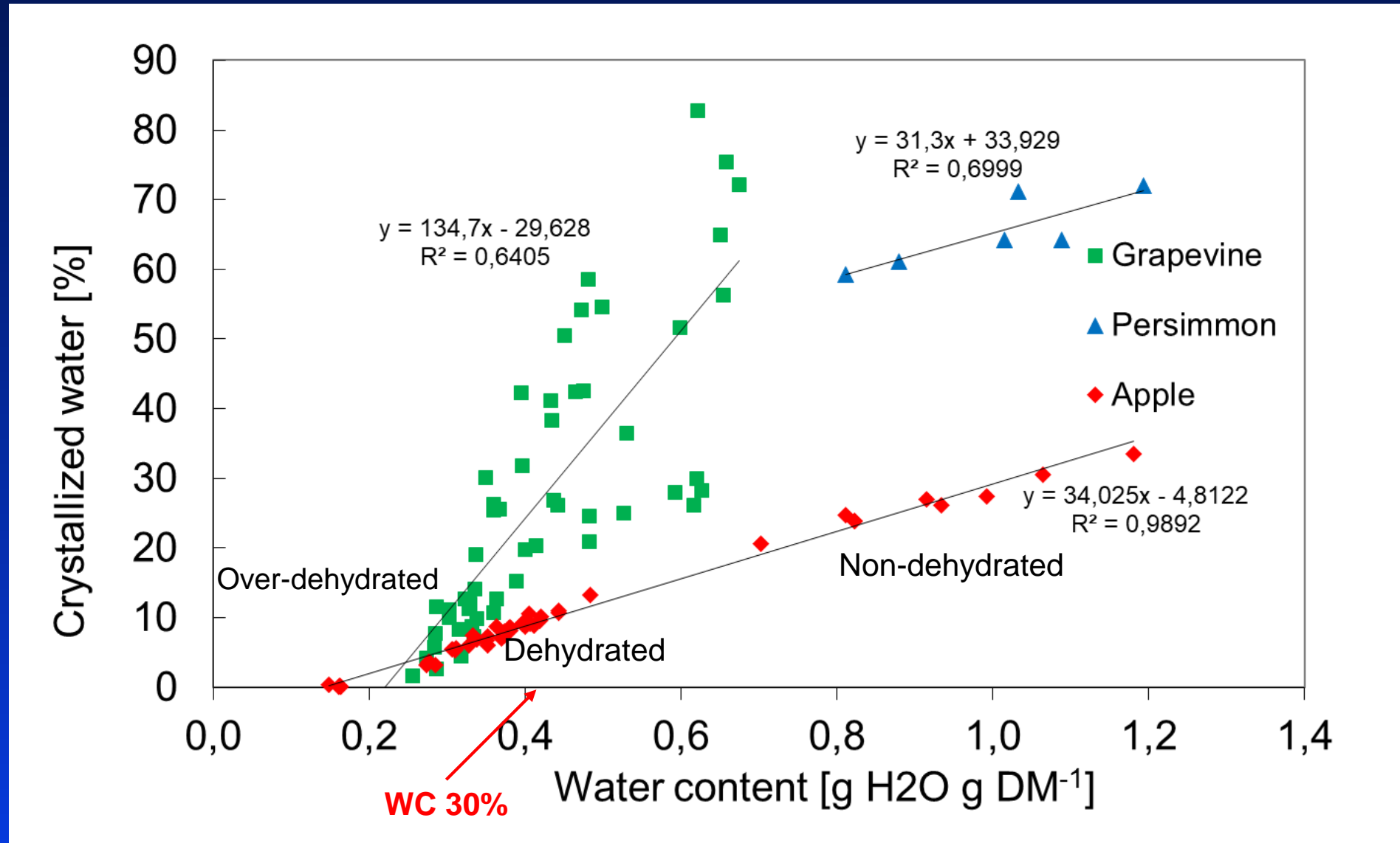


Amount of crystallized water in dormant fruit tree buds

DSC melting peak integration - warming

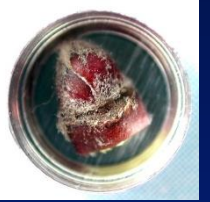


DSC pan with apple bud

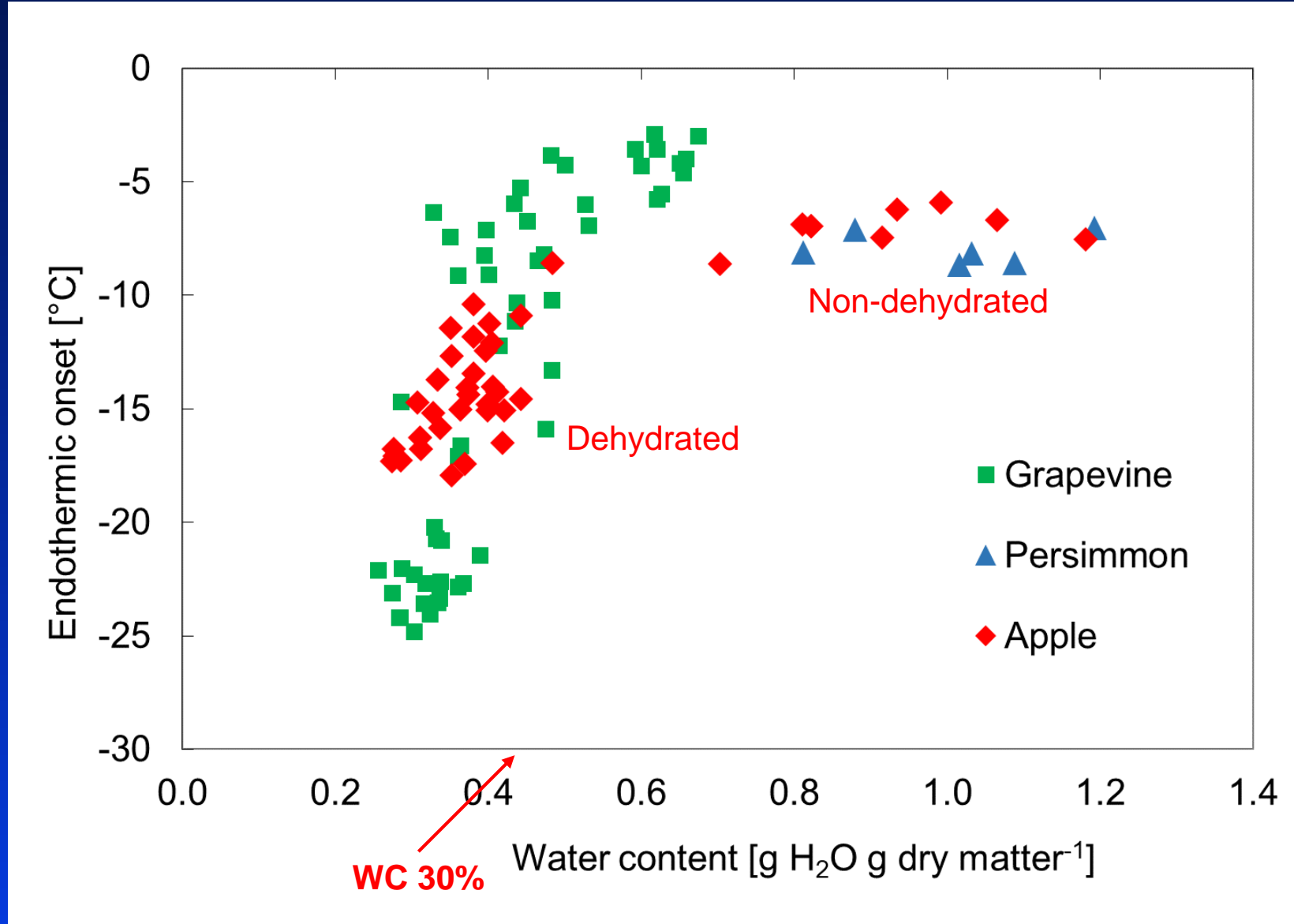


Onset of crystallization peak in dormant fruit tree buds

DSC melting peak - warming

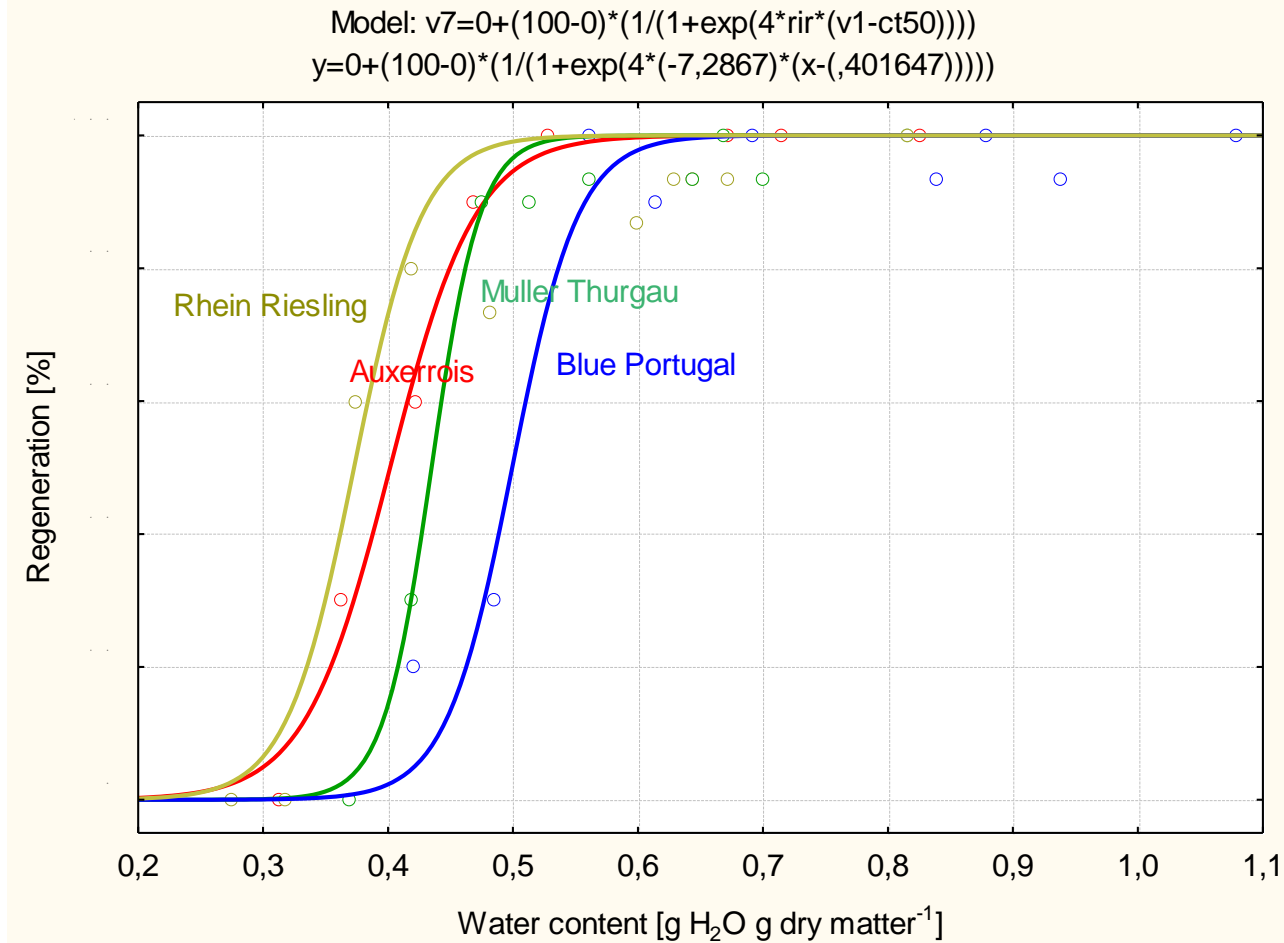
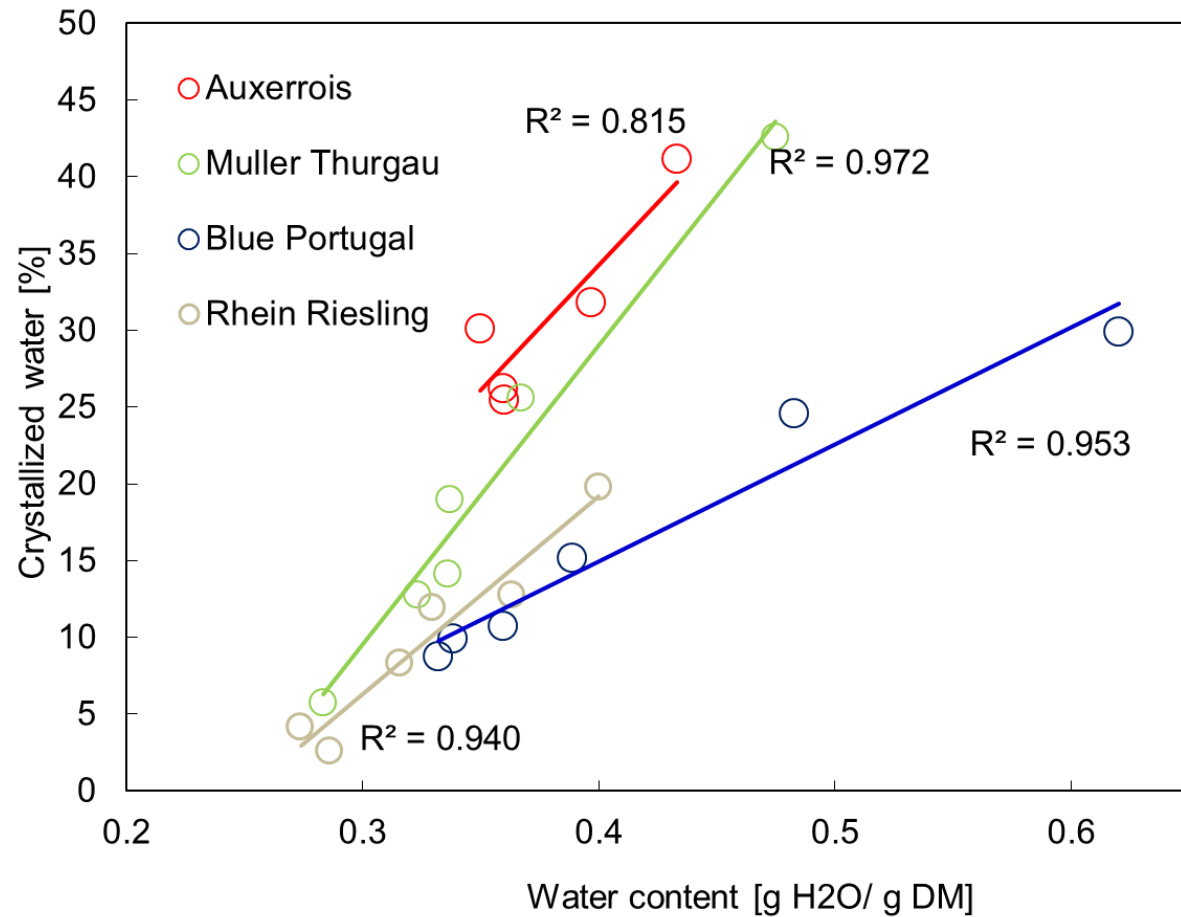


DSC pan with apple bud



Variety differences

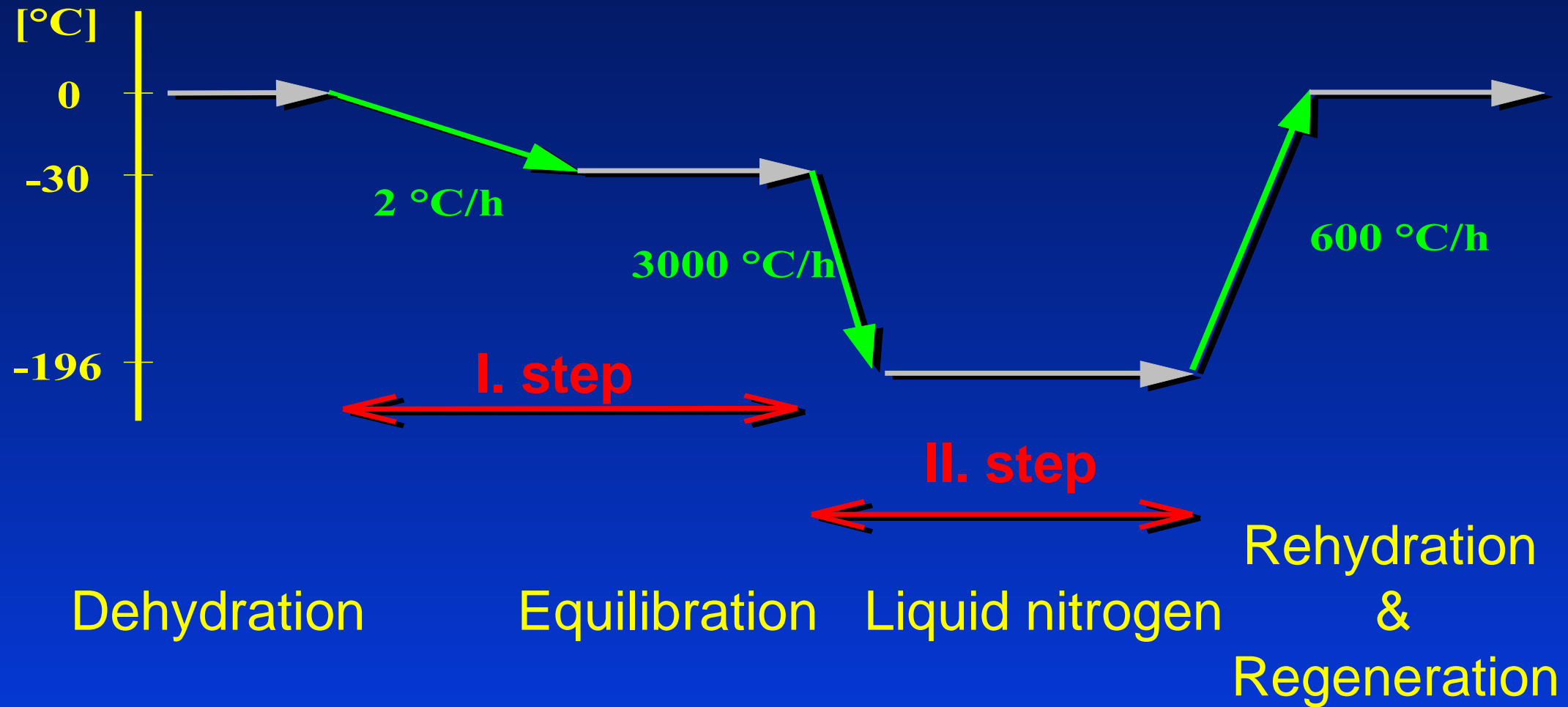
Grapevine dormant buds



PRACTICAL APPROACHES



Scheme of two-step cryopreservation



Two-step cryopreservation

1st step

- Apple – from -5 °C to -30 °C (1 °C/h)
- Pear/apricot – from -5 °C to -25 °C (1 °C/h)
- Other resistant species – as apple
- Annealing for 24 h

2nd step

paper boxes placed in LN vapours, after 45 min into the Dewar flask



Pretreatment

- Cuttings – 35 mm at outside conditions/chest freezer
- Dehydration & cold hardening – frost dehydration in packs at $-4\text{ }^{\circ}\text{C}$
- Period (from one week to more than 8 weeks)
- Water content (apple - 30 %, pear 35 – 40 %, apricot 40 %, ...) control weighing



Dormant buds - Encapsulation

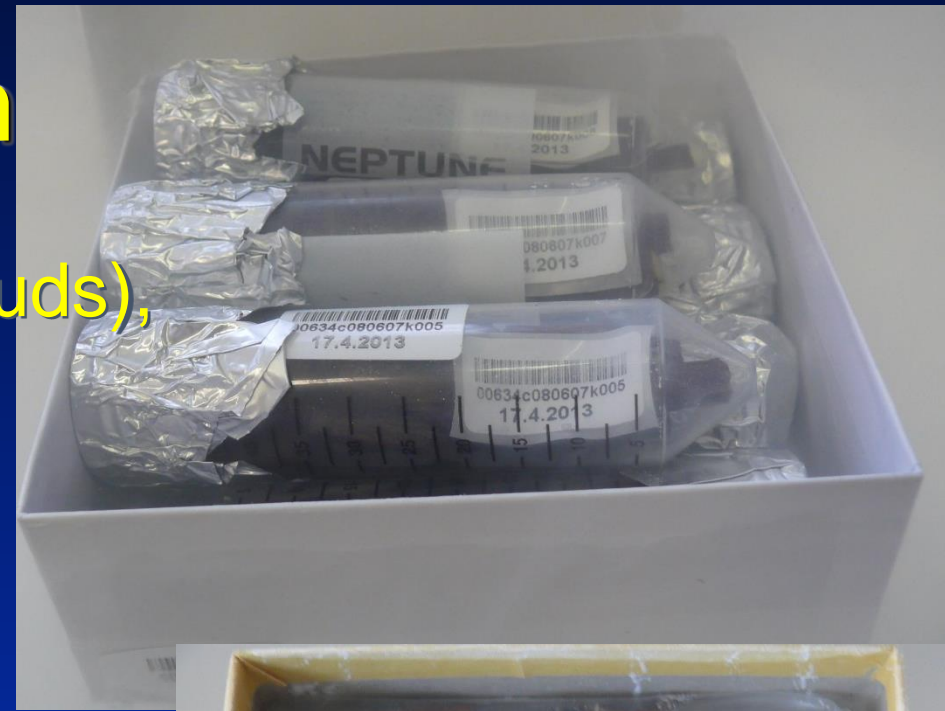
Untreated buds

Encapsulated buds



Storage system

- Cryovial – plastic centrifugal tube (20-25 buds), 100-120 LN storage, LN control, 1st step control,...
- Labeling – bar code,...
- Handling – at low temperature (freezer)



Regeneration - evaluation

- Visual evaluation, regrowing in water, grafting on rootstock (potted plants, freely growing plants)
- Chip budding in orchard – two buds per plant
- Timing
 - Spring grafting - end of May - beginning of July
 - Grafting on sleeping bud (end of July – beginning of August)
- Evaluation – after 100 – 130 days



Warming and rehydration

- Warming
 - Slow - cold chamber at 4 °C overnight
 - Fast – water +38 °C
- Rehydration
 - moist white peat,...
 - cold chamber at 2 - 4 °C for 14 days



Regeneration – visual evaluation



Currant



Regeneration – grafting (Chip budding, cleft grafting)



Regeneration – grafting

(evaluation of chips before grafting)



Apricot



Apple



Apple



Accessory buds



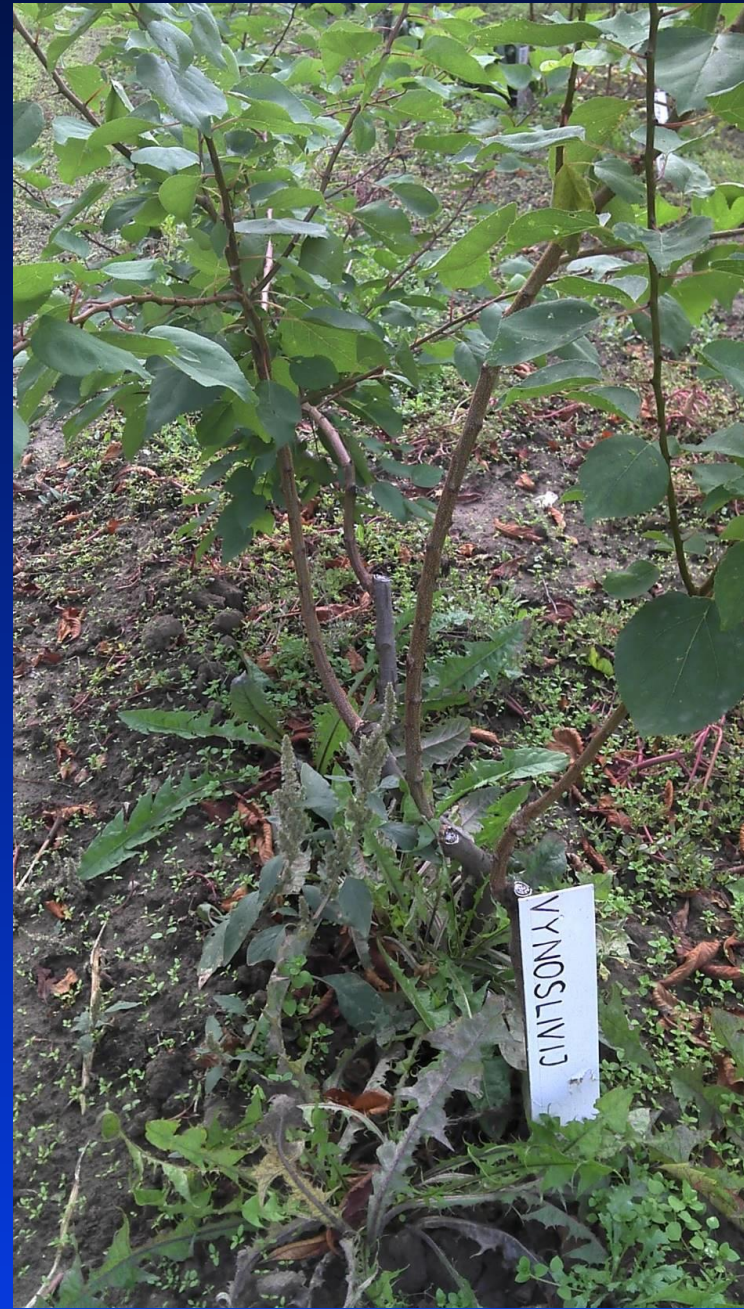
Dead buds



Hale damage



Apricot



Apricot



Apricot



Regeneration – grafting/sprouting Blue honeysuckle



grafting

sprouting



Regeneration – *Ribes*



Ribes petraeum



Ribes nigrum



Regeneration – rowan *Sorbus acuparia* 'Granatnaja'



Regeneration – raspberry 'Willamette'



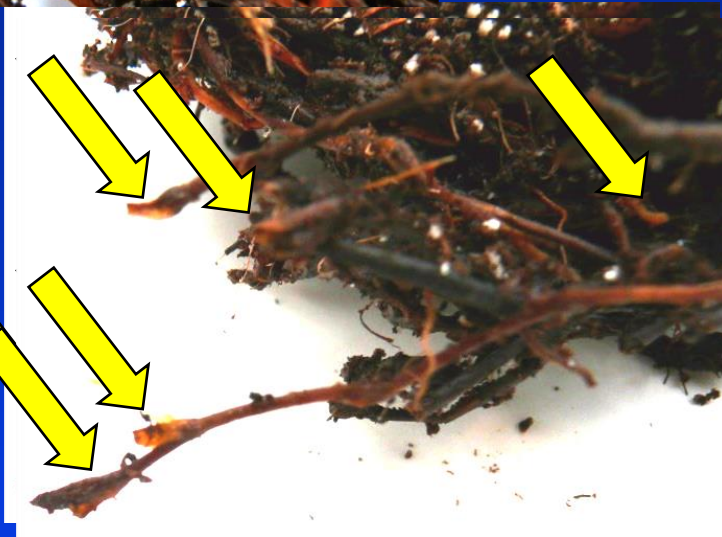
-30 °C



LN



Cryo-regeneration – *Rubus chamaemorus*



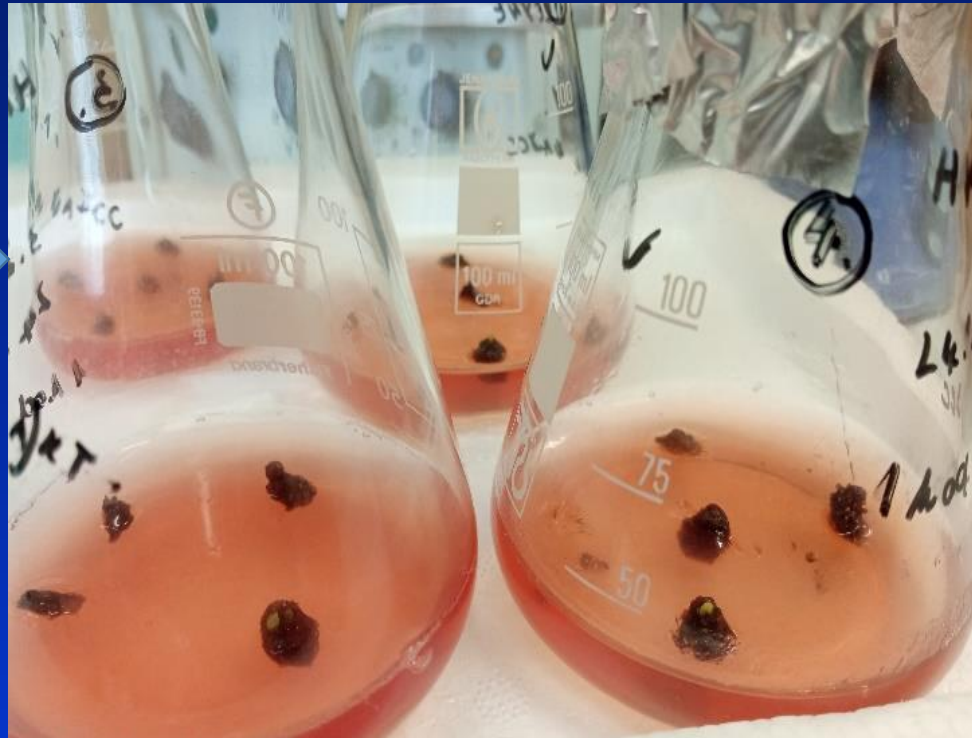
In vitro escape

Regeneration of cryopreserved buds through *in vitro*

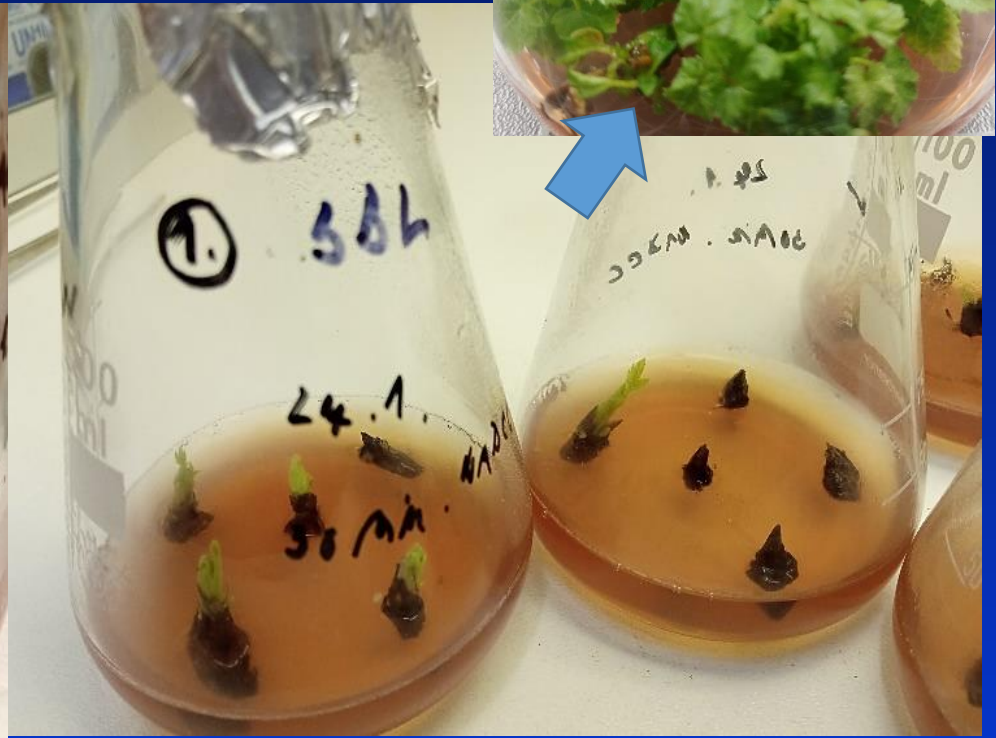
Raspberry 'Sanibelle'



Dissected bud from cryopreserved nodal segment



10 days

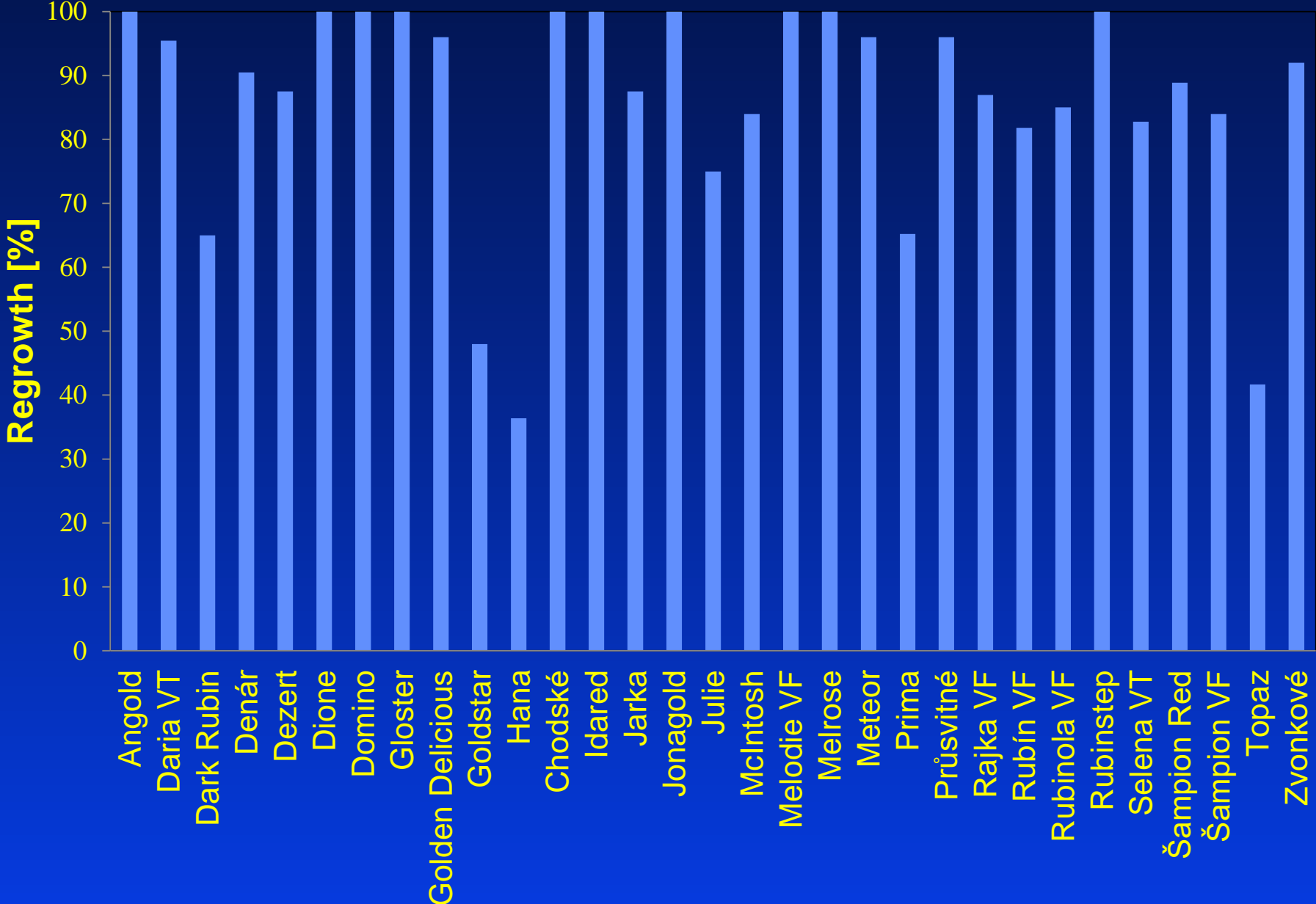


13 days

after introduction in vitro after cryopreservation

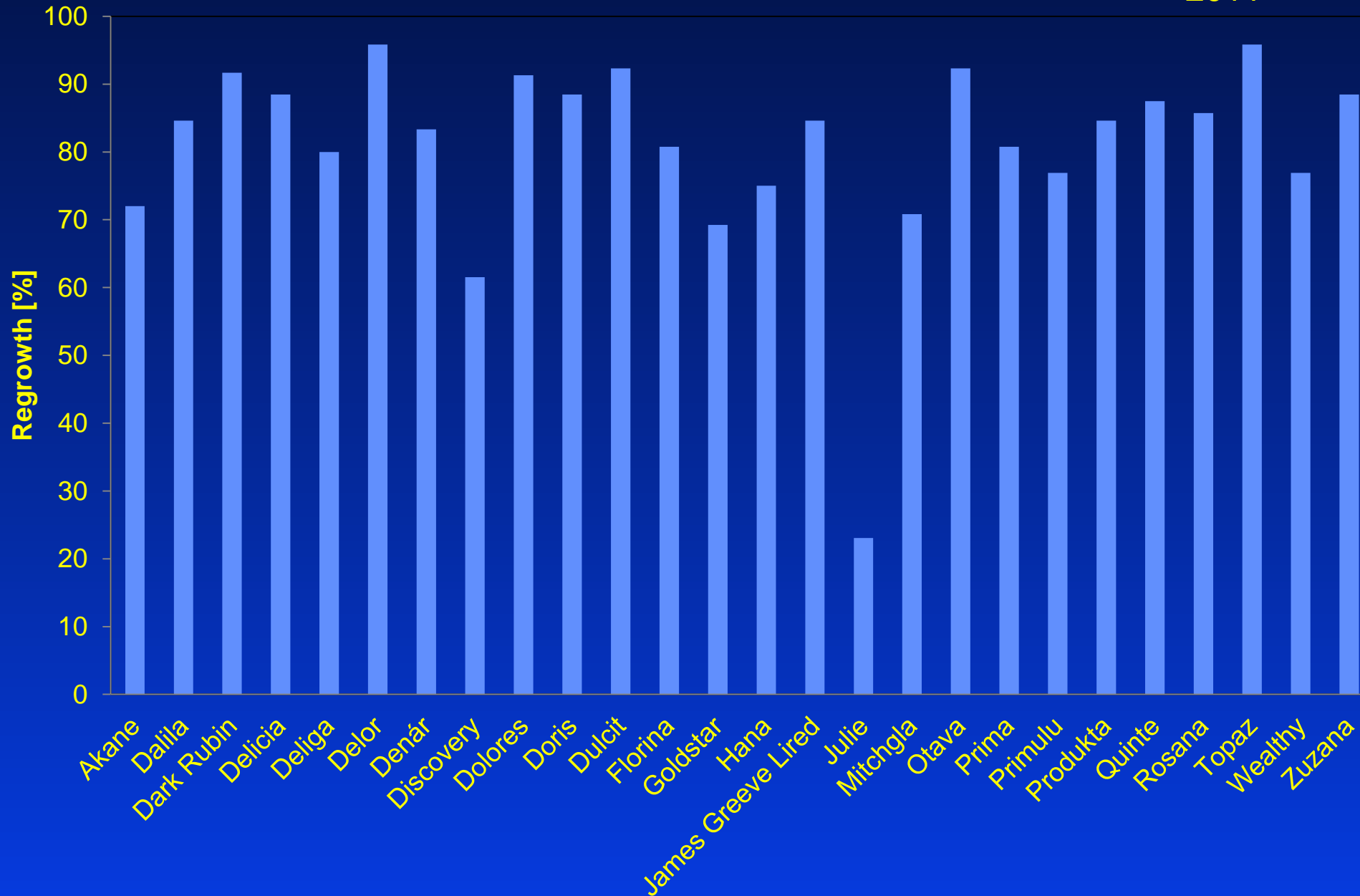


Apple cultivars - regrowth after LN ■ 2010

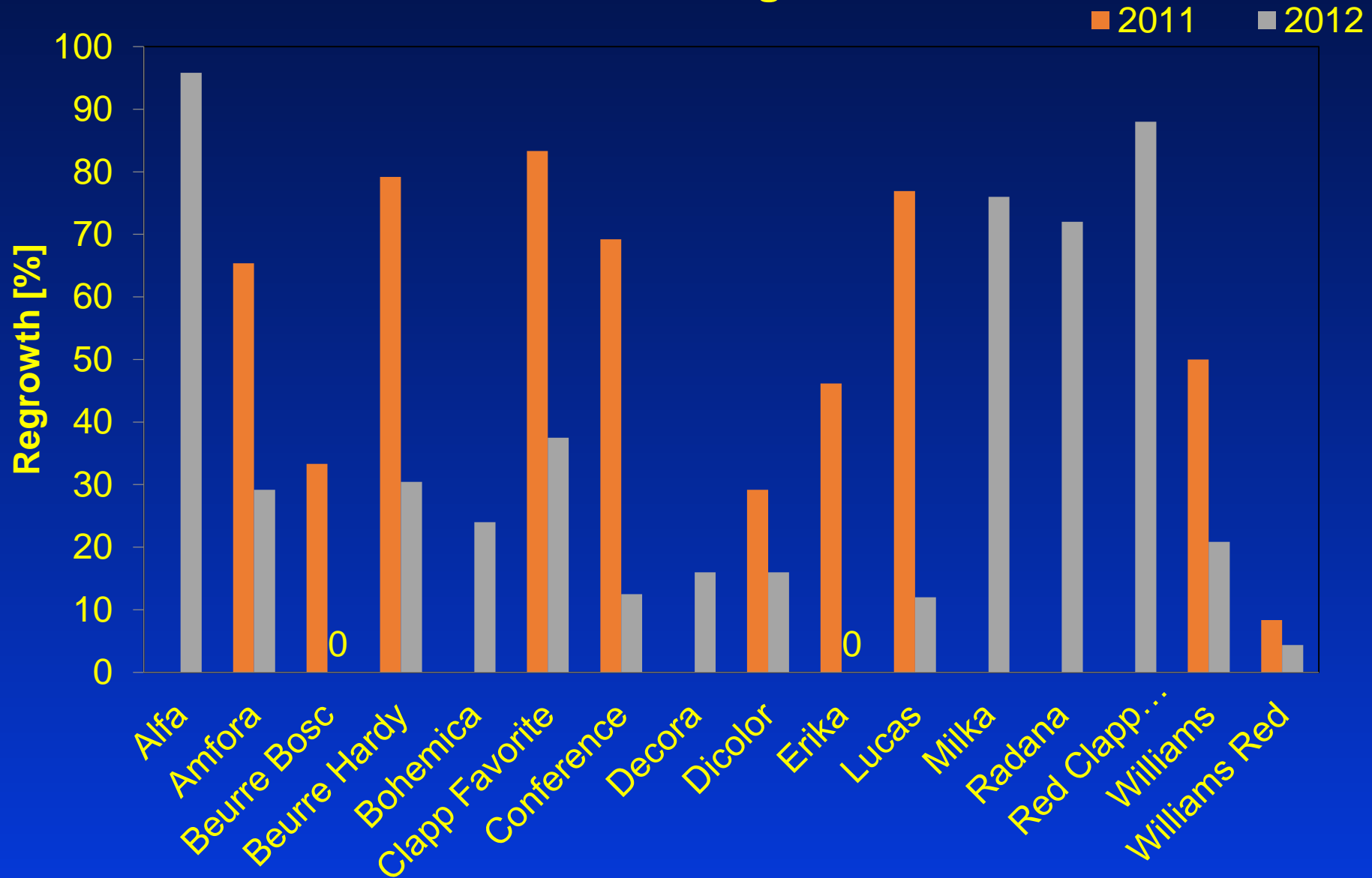


Apple cultivars - regrowth after LN

■ 2011



Pear cultivars - regrowth after LN



Cryobank (dormant buds)



			Regeneration	Water content
	year	n	[%]	[%]
Apple	2010	34	87±16.8	29.1±1.58
	2011	26	81±14.3	30.7±1.28
Pear	2011	10	54±23.6	40.1±1.46
	2012	16	33±30.7	34.3±3.04

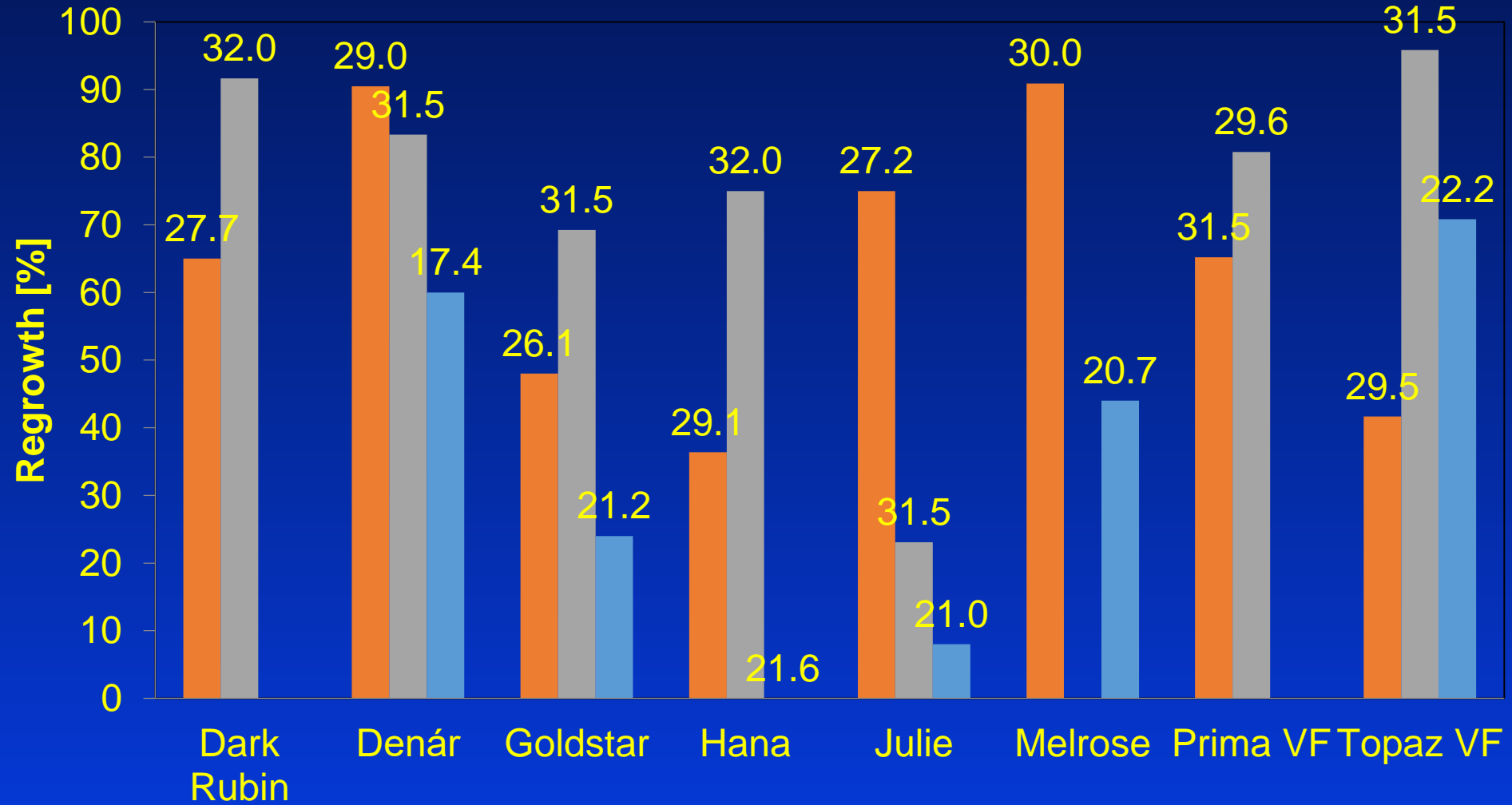
* 50 apple cultivars in total in cryobank as dormant buds

** 13 pear cultivars in total in cryobank as dormant buds



Apple cultivars - regrowth after LN numbers - Water Content [%]

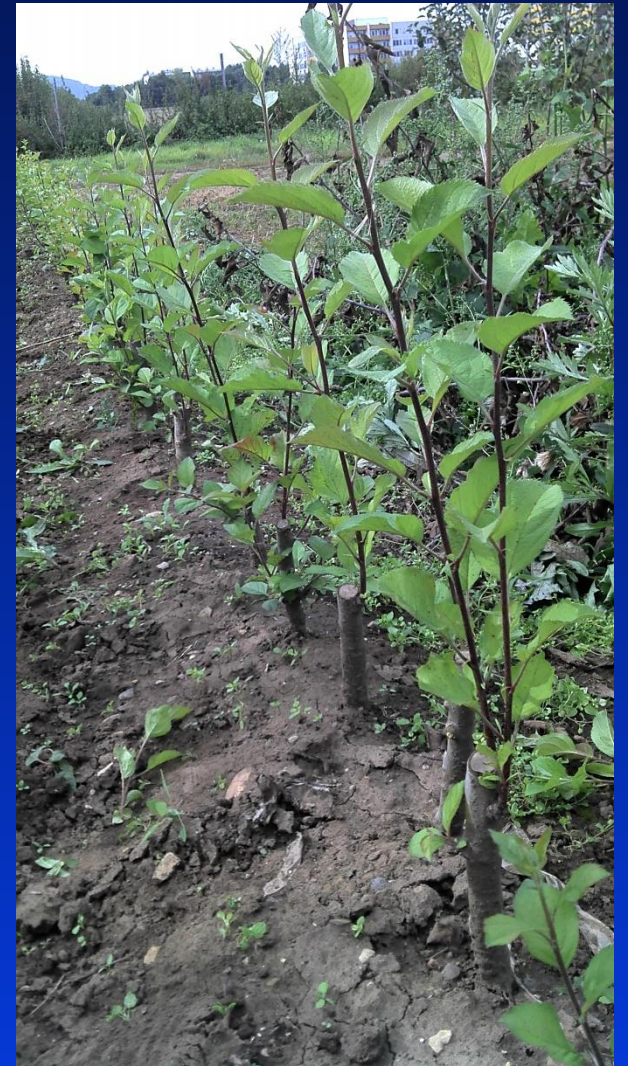
2010 2011 2012



Czech Crop Cryobank - Current state

Dormant buds

- Apple - 56 accessions
- Pear – 16 accessions
- *Malus* spp., Apricot, Blue honeysuckle



Harvest in the Cryo-orchard, CRI (2010 est.) (240 trees 50 varieties)



2018



PRACTICAL TIPS



Sampling/preparation



AW – water activity measurement

Water Activity Meter HP23-AW-A (Rotronic, Switzerland, HW5 software)



Dehydration freezer

- Low end regulator Honeywell PCR110



Two-step cryopreservation freezer

- Programmable regulator
Honeywell
DCP100
- Important: slow
cooling speed!



Table 5-3 Programmer Parameters - Functions

Parameter	Five-digit {DATA} Element		Dependent Upon:
	Adjustment Range	Decimal Point Position	
Segment Number	0001 to 0016	0	Program Number
Final Setpoint Value	Input Range Min. - Input Range Max.	As per input	Program Number Segment Number
Segment Time/ Ramp Rate	Time Mode: 0000 - 9959 (2 right-most digits must not exceed 59) Rate Mode: 0000 - 9999 000n - Join to Program n (n = 1 to 8), 0009 - Repeat segment, 0010 - End segment	2 As per input 5 5 5	Program Number Segment Number



Storage systems





Thank you for your attention

