



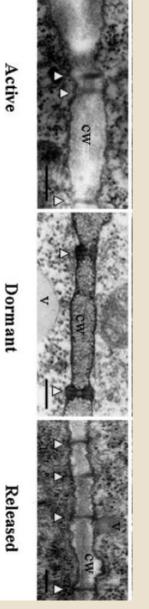
Dormancy-Track

Could initiation and progress of bud dormancy in temperate deciduous trees be tracked using water isotopes?

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- During autumn temperate deciduous trees cease growing and loose their leaves
- They enter a state of dormancy, where the hydraulic conductivity between twig and bud is restricted (Rinne, Kaikuranta et al. 2001)
- Callose and protein containing plugs (dormancy sphincter complex) contribute to this isolation (Rinne, Welling et al. 2011)
- Callose production and degradation is involved in many different biochemical processes (De Storme and Geelen 2014)
- Is dormancy release one of them?
- Idea:
 - Dormancy sphincter complex prevents symplastic water flow at highest dormancy depth.
 - > Increasing chilling decreases dormancy depth by decreasing symplastic isolation



Rinne,

Kaikuranta

et al.

2001



Aim

Tracking of symplastic isolation between twig and bud using water isotopes

- 1. Quantification of timepoint when buds become isolated from the rest of the plant and when the connection is restored
- 2. Is there a difference in the timing of dormancy induction and release between species?
- 3. Is water influx in buds correlated to dormancy depth?

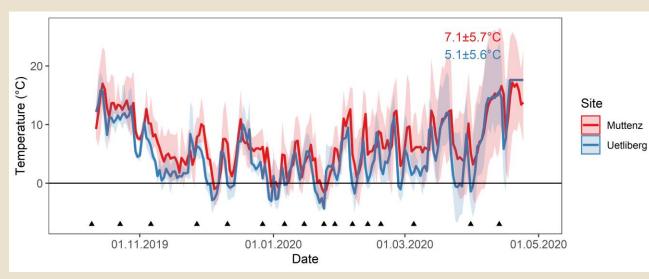






Method

- 2 sites with a temperature difference of \sim 2°C •
- 5 different species .
- 5 individuals (replicates) per species and • elevation
- ~15 sampling campaigns during winter • 2019/2020 (biweekly, weekly)









Method

- Sampling of 2 twigs per individual and campaign
- Recutting of **twig 1** before placing into deionized water (for dormancy depth)
- Recutting of twig 2 in about ten pieces (~8 cm long) each containing one single bud at the uppermost part (for water uptake)
- Placement of **twig 2** pieces into labelled water $(\delta^2 H \sim 2000)$ for at forcing conditions for 24 h



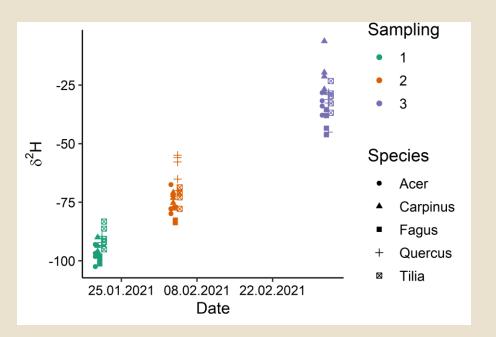
- Cutting of buds of **twig 2** 2 mm above the base of the bud
- Water extraction for isotope analysis and determination of water content

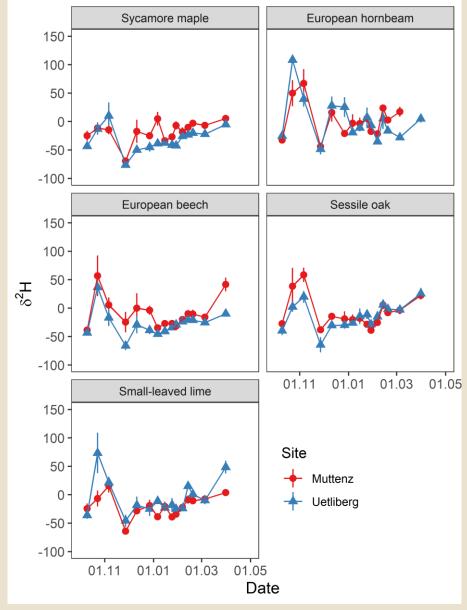




Method

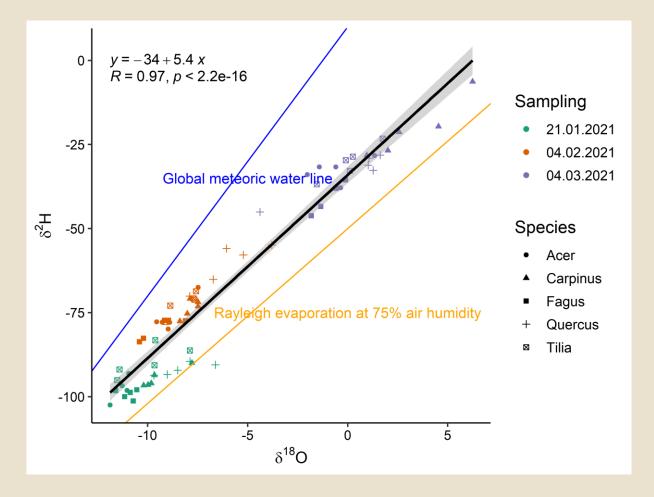
- How to calculate water flow from twig into bud from $\delta^2 H?$
- Naive assumption: Bud-water δ^2 H stays the same for the entire winter dormancy.
- **Reality:** Bud-water $\delta^2 H$ changes over time







How to take into account fluctuations in bud-water δ^2 H?

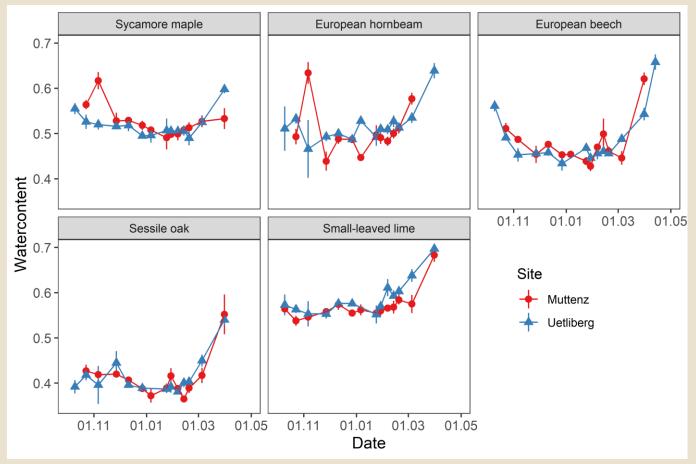


- Calculation of natural δ^2 H using δ^{18} O
- Expectation: the relationship between bud-water δ²H and δ¹⁸O could be explained by the global meteoric water line
- **But:** Water isotopic composition of the water inside the bud equals rayleight evaporation at 75% air humidity
- Do buds loose water by evaporation during winter?



Results: Bud-Water content during Winter

- Bud-watercontent during winter
 dormancy depends on species
- Change in bud-watercontent during winter depends on species
- Higher symplastic isolation during winter for beech compared to lime?

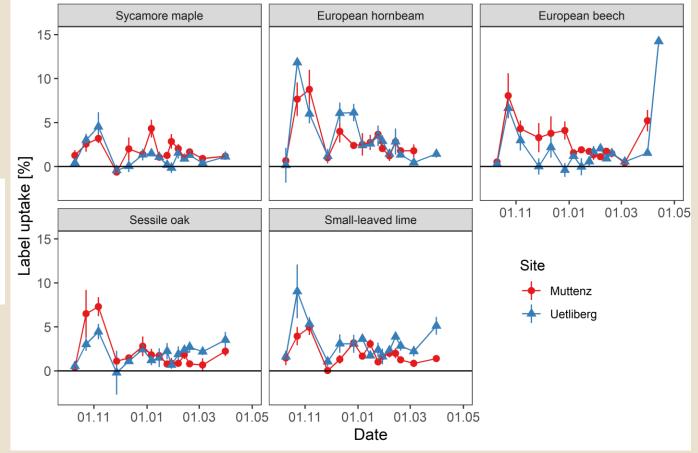




Results: Label-uptake during winter

- High label uptake at the beginning of winter dormancy and at the end
- No progressive restoration of symplastic isolation during winter

Is air temperature the most important driver for the symplastic isolation during winter for all species?

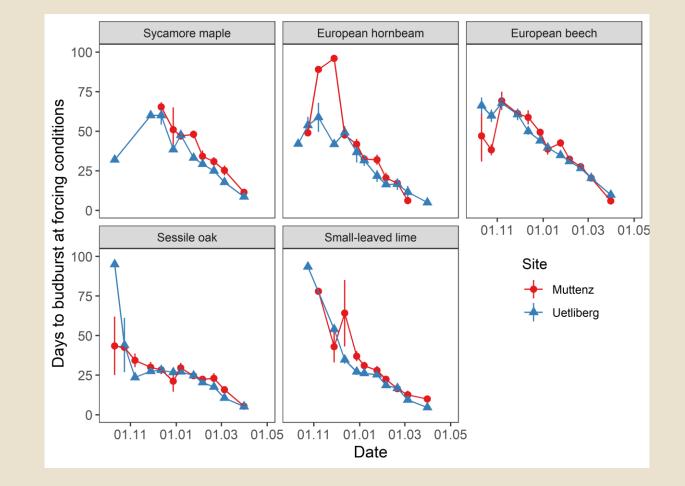




Results: Label-uptake and dormancy depth

- Dormancy depth decreases with increasing chilling for all species
- **But:** No relationship between labeluptake and dormancy depth
- Quantity of water flow between twig and bud is not a good indicator of dormancy depth

What is the plant biological mechanism of chilling and chilling accumulation?

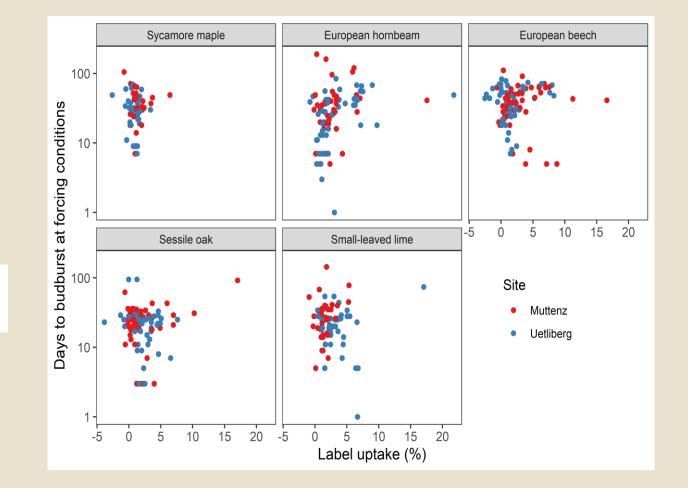




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Conclusion and outlook

Objectives:

- 1. Quantification of timepoint when buds become isolated from the rest of the plant and when the connection is restored
- 2. Is there a difference in the timing of dormancy induction and release between species?
- 3. Is water influx in buds correlated to dormancy depth?



But how can we determine dormancy depth from molecular measurements?

- Idea 1: Exploring the ABA signaling pathway: (See poster S7.P9 of Bénédicte Wenden et al.)
- Idea 2: Explore mechanisms driving frost hardiness: (See Kovaleski, A. P. 2022)

