

Chilling paper(s) notes

Lizzie, Fredi, Jonathan Auerbach on some

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Contents

1 Next steps

1. Develop a good diagram and understanding of the callose model... now
2. Keep meeting with Auerbach – schedule when all three can make it – hopefully he comes up with what we can say, can't say, assumptions that would help
3. Fredi is reading the freeze lit; Lizzie will put name of refs in a new issue (and share OSPREE folder) – see *When reading papers* below
4. Lizzie is reading the old model literature to write up the history and get assumptions (already has most files, need to read and organize notes) – *When reading papers* below
5. Not yet done – maybe read dormancy induction studies in OSPREE
6. Meetings scheduled! First one is Leap Day – 29 Feb 2024!

When reading papers

1. What they think of and how they deal with endo/eco versus chill/force (versus just rest)
2. How they % versus days?
3. What they could allow us to assume ... what assumptions they support or violate?
4. Which species

2 Current outline

Current models of spring tree phenology are non-identified and thus semi-imaginary
What do we want to take away from this paper?

1. Recognize current chilling models are extremely flawed ... based on fruit tree crops, non-identifiable and few studies conclusively show two-stage for many species (we think)
 2. Need to do better experiments! (And build fewer chilling models.)
1. Introduction

- (a) We all think dormancy is critical to many forest trees (and thus climate change), but is dormancy real? (Baby: Good forecasts)
- (b) Concepts of dormancy: para, endo, eco (Lang ref)
- (c) Maybe review quickly evidence against in (and maybe for it) – Cook et al. ref (Fred's ref)
- (d) Is it real or just helpful? – Warewolf!
- (e) Here we ... briefly review two-stage model (maybe where came from) and its general application to forest trees), lay out how it's actually non-identified given (all?) current data, review critical evidence/info we have that could build towards an identified model, especially given new hope to measure the mechanism of endodormancy (callose/hormones)

2. Review of two-stage model

- (a) Logic of the two-stage model
 - i. Dealing with cold climates (fit in continentality here?)
 - ii. What would evolve to avoid frost? (and still compete for resources)
- (b) History of the two-stage model (and related models) – they % more than time to BB
- (c) Application of two-stage model to forest trees
 - i. How it's been applied
- (d) New evidence for two-stage (callose, hormone) – work through what the current callose model predicts, and doesn't and also how well it fits to two-stage
- (e) How good is this model?
- (f) Well, it's non-identified ... You can't estimate that many parameters
- (g) Because it's non-identified it's lead to a whole slew of models – many, many models! But we can't actually identify the parameters.

3. Critical evidence to build a better model (what assumptions can we make)

- (a) Time matters ... is it only time, no also time at different temperatures: < 0 , 0-10, > 10
- (b) Temperature ...
 - i. Optimal chill – evidence for this – % versus time to BB: what tells us what?
 - ii. Subzero temperatures
 - iii. Intermittent warm periods
 - iv. Fall temperatures (don't forget we have OSPREE papers on induction temperatures)
- (c) And photoperiod
 - i. at induction
 - ii. During ...
 - iii. In releasing endodormancy (check OSPREE papers with non-0 chill photoperiods)
- (d) Species diversity...

- i. Crops vs. wild
 - ii. Species diversity
 - A. Assume universal model with different parameters for each species or population (mention parameterization for individual species), kind of weird, no?
 - B. Seeds don't have a universal model
 - iii. Subtropical trees – Should they belong to the same model?
 - iv. where this subtropical evidence fits and how could we start to build a framework to predict different models for different sets of species
 - v. Population diversity (including continentality – find the Doug fir paper on chilling being higher in coastal areas)
 - vi. How good is the evidence for population differences? Chilling differences at site; induction temperature differences (and what if photoperiod matters?)
4. Future directions ...
- (a) Acknowledge chill and force treatments do not usually measure chill and force
 - (b) So we need better experiments
 - i. More work with molecular pathways at the same time you do chilling/forcing experiments
 - ii. Maybe more with % budburst or otherwise looking for evidence of endodormancy
 - (c) Critical experiments to deal with non-identifiability that we can do now ... if we have figured any of these out
 - (d) Jump on breakthroughs in callose/hormones to improve experimental measurements of endodormancy – check if it is similar across species

3 Meeting notes

3.1 8 December 2023: Lizzie meets with Loren Riesberg

Full notes in my gray and green notebook, but some major points to remember here:

- I started by describing general models of chilling and forcing we have and Loren quickly said that it indeed sounded like methylation, and methylation patterns would be a good way to tell these models apart.
- Good work by Caroline Dean (Innes Centre, UK) on *Arabidopsis*. Good recent talk here: <https://www.youtube.com/watch?v=N4qnj7cSZdo> ... he suggested to then look at what papers have cited Dean work in Populus and peach. This work is all about silencing that leads to one step (plant shutdown) and silencing that then leads to growth.
- He thinks Dean work shows that prolonged and lack of hot spikes promotes FLC
- Read up, then have a call with Dean, and then a call with a Populus group.
 - FLC (MADS box) represses (silences) genes that cause flowering: See around minute 12 she shows FLC slowly being turned off during the winter.
 - VIN3 is the cold-regulated protein
- ‘Epigenetic clock’ that goes forward and backward
- He expected subzero temperatures do not do anything, but could ask Rob’s opinion (IMHO, it makes sense to me that subzero would not matter to dormancy as it doesn’t seem to give much extra info or would be needed to do **do** anything: if you build callose during cool temperatures and do nothing below zero that works! Why do anything sub-zero for a process like dormancy?)
- He expects callose could be built and degraded throughout the winter (see below, goes with high temperature negation model)
- I asked about evidence that dormancy induction temperatures matter and he replied that he expects some plants start dormancy processes, then stop, maybe go backward, start again ... and that could look like the idea that dormancy induction temperatures matter.
- For modeling, we really need to know how fast is regression compared to accumulation – see if Dean has looked at this.

My thoughts after this meeting:

- Most papers I have looked at seem to be finding orthologs for Dean’s work.
- Loren expected (and Dean seems to expect/find for populations) differences in what temperatures matter across populations and species
- Models ...
 - Molecular results do support idea of accumulation of cool temperatures.

- Chilling probably does not start as soon as we think it does (for example, August or September). It starts once there is transcriptional shutdown, and callose built ... though I am not sure anyone has shown how long these processes to take, which would be good to know.
- In Dean talk around minute 23 she says that VIN3 is very slow ... she says it takes weeks and weeks to silence. VIN3 takes 6 weeks of cold. So the cold accumulation is slow at the molecular levels.
- Models with high temperature negation may make sense.
- Parallel model where plants are gaining chilling and forcing at once seems wrong. Since there is callose that needs to be unblocked for budburst to start, and we know there genes turned on for making callose, and turned off to degrade it, this seems a bad model.

3.2 4 December 2023: We meet again on this!

In the morning we had a big conversation about what is the model for leafout, especially for ‘chilling.’ Questions we ended up with:

1. Dormancy depth as measured by forcing units needed (days at 20°C in Fredi’s 2021 paper for example) – is that an okay response variable or do we not understand forcing enough and days should be the response? If so, how do we model it altogether?
2. Should we model % or days or % over days?
3. Also, exciting short conversation on bet-hedging and leafout!
 - (a) Should we model buds as cohorts and include variability as an expected response? (**Bet-hedging and buds**) Could think of cohort as having varying dormancy depths and thus needing different forcing units
 - (b) In Fredi’s experiment, some species never burst much about 50-60% ...
 - (c) If bet-hedging is happening but we assume % budburst is related to dormancy depth, we may confuse the two completely.

Then we chatted with Jonathan Auerbach – notes mainly in my green/gray notebook but a few here:

1. How to model experiments and observations together (Lizzie’s eternal dream)
2. Renewal theory is basically the bucket model
3. What could we hope to do?
 - (a) Figure out what our current experiments DO show. For example, can we show that chilling affects forcing? (Can we show it’s not just time, but that temperature matters?)
 - (b) What can we say about chilling and forcing with current data? Given this is probably not much given identifiability issues ...
 - (c) What assumptions can we make that would allow us to say more?

- (d) What are the critical experiments that would test assumptions or really advance things

In the afternoon we brainstormed: **Points to cover in paper and/or /disturbing problems/issues:**

1. Is the model two-stage or parallel (or both, but depends on species ID)?
2. We cannot (and do not) fit the experimental and observational data together.
3. Models are basically made-up based on old studies ...
4. Where does evidence/theory come from for current models of chilling?
5. Models are non-identifiable
6. Hardiness vs. dormancy
7. Temperature fluctuations
8. Influence of time without any other effects
9. Photoperiod – during ‘chilling’ and during ‘forcing’
10. List out big things that matter and maybe smaller things that matter
11. Transition time from endo to ecodormancy – is it instantaneous or gradual? What do we know from callose?
12. What is the callose model? What temperatures is it degraded at? When is it built? What exactly is it blocking between cells (hormone etc.)? – How does it compare to the two-stage and parallel model?
13. Hormones and dormancy?
14. Molecular evidence etc.
15. Corollaries with seeds
16. Dormancy depth (forcing needed after putting in warm temperatures) since budset through to next budburst
17. GDD model is based on development – but do we know if this is correct? It may be more like a timer that also runs (differently for each species). What is structural growth? Maybe none of it? GDD is from crops and is mainly structural growth, even though we don’t think structural growth is happening during budburst?
18. Basically, what’s chilling? And what’s forcing?
19. What we don’t know with chilling
 - (a) What temperatures accumulate chill? Can it be really low? Can it be really high? What do freezing temperatures interrupting this do?
 - (b) Can accumulated chill be negated?

- (c) Is it just time?
- (d) Just to confirm: Chilling happens below 10 C only/mostly?

20. Relevance of chilling in subtropical trees.

We need to. ...

1. Understand the progression of the major old literatures that lead to the Utah model and the other model (Fishman? This is precursor to dynamic chill?).
2. Confirm how these models were extrapolated to forest trees (or at least compare them to the current forest tree models) – we could just ask Isabelle about this and check Harrington papers and Murray et al. 1989
3. **Remember** to Never re-read chill models, just read old notes I have
4. Divide up lit review tasks ...
 - (a) Fredi does all OSPREE papers with negative chill or freeze... (Lizzie sends them to him, see getchill.R)
 - (b) Somebody does dormancy induction OSPREE papers
 - (c) Lizzie does the old modeling papers

Tomorrow! (4 December 2023)

1. Arrive having read Isabelle's 2016 paper
2. Make a broad outline ... some points already
 - (a) Callose model vs. the current models (Lizzie has old notes, see notes within `chillingrefs_holidayedi`)
 - (b) What determines dormancy release?
 - i. Time alone
 - ii. Time at different temperatures: < 0 , $0-10$, > 10 ...
 - iii. What do high temperatures do to 'chilling'
 - (c) % versus time to BB: what tells us what?
 - (d) What are feasible models?
 - i. What would be the best model?
 - ii. What do what know about what plants can measure?
3. Officially divide up tasks and decide when feasible/best to do them
4. If time allows, work on callose model/papers

3.3 Thinking ahead on 3 December 2023 before meeting tomorrow

I think a good lit review could be in order for this paper ... but we'd need to think hard about how to do it. We'd likely want to include endo and ecodormancy (how well they measured it and included it ...). Then I just worked up `chillingrefs_holidayedition.pdf`

3.4 Notes from very brief chat with F. Baumgarten on 1 November 2022 about writing a concept paper on chilling

Next steps:

1. Fill in outline, esp. the what we know on chilling
2. Pull the OSPREE papers and compare across the different questions (subzero temps, intermittent warm etc.) – are there any consistencies?
3. Read all the old papers I have pulled!
4. Read all our old notes, organize ...

Outline... Coming out of the dark ... the critical role of photoperiod in chilling

Fig 5.1 — Baumgarten discussion

1. What is the known (and possibly known) biology of of chilling?
 - (a) Optimal chill
 - (b) Intermittent warm periods
 - (c) Subzero temperatures
 - (d) Fall temperatures
2. What are the chilling models?
 - (a) Utah
 - (b) Chill portions ...
 - (c) Adapting to each species' optimal chill (and Lizzie's worries over this)
3. So what do we really know (esp. maybe to improve chilling models or at least approach them with caution when we interpret them) ... What are the biases/uncertainties given all this? What is definitely known?
 - (a) If we don't know chilling then we have issues with forcing also ...

3.5 Notes from whiteboard (December 2023) written up in April 2024

Things we really need to know to merge chill/force models and molecular/physiology understanding:

1. When is callose built and for how long is it active?
2. Callose: Is it partly or fully blocking plasodesmata?
3. Callose: Is it reversible?
4. So, is endodormancy when callose ...
 - (a) is already built and not degraded?
 - (b) callose is being degraded (which would fit with 'chilling')?
 - (c) in some feedback loop (constantly built)?
 - (d) built and degraded but enough to block cells?
5. So, when chilling have an impact?